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Microbiology & Pathology

Highlights

- Escherichia Coli Infection
- Patterns of Thyroid Lesions
- Primary Pre-Sacral Carcinoid
- Capsules containing L-Cysteine

Discovering Thoughts, Inventing Future

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Preparation of Capsules Containing L-Cysteine with Melting Dispersion Cooling Method

By Satoko Mesaki, Yoshinari Taguchi & Masato Tanaka
Niigata University, Japan

Abstract- It was tried to prepare the capsules containing L-cysteine with the melting dispersion cooling method. Tripalmitin was selected as the shell material in order to keep out water and a few fatty acid esters such as ethyl laurate, ethyl stearate, ethyl myristate, ethyl oleate, ethyl palmitate and bees wax were added in the shell material as the modification materials in order to improve the water proof of the capsule shell. Furthermore, the capsules were coated by the coating materials such as oleic acid, ethyl oleate, triolein and ethyl laurate. It was investigated how the concentration of oil soluble surfactant and the combination of the shell material with both the modification materials and the coating materials affected the characteristics of capsules such as the content and the release feature of core material, the water proof and the swelling degree of capsules. With increasing the concentration of oil soluble surfactant, the released ratio decreased, become minimum and then, increased. The content could be increased by addition of modification materials.

Keywords: L-cysteine containing capsules, tripalmitin, melting dispersion cooling method, release controlling, fatty acid esters.

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PREPARATIONOFCAPSULESCONTAININGL-CYSTEINEWITHMELTINGDISPERSIONCOOLINGMETHOD

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Preparation of Capsules Containing L-Cysteine with Melting Dispersion Cooling Method

Satoko Mesaki [α], Yoshinari Taguchi [σ] & Masato Tanaka [ρ]

Abstract- It was tried to prepare the capsules containing L-cysteine with the melting dispersion cooling method. Tripalmitin was selected as the shell material in order to keep out water and a few fatty acid esters such as ethyl laurate, ethyl stearate, ethyl myristate, ethyl oleate, ethyl palmitate and bees wax were added in the shell material as the modification materials in order to improve the water proof of the capsule shell. Furthermore, the capsules were coated by the coating materials such as oleic acid, ethyl oleate, triolein and ethyl laurate. It was investigated how the concentration of oil soluble surfactant and the combination of the shell material with both the modification materials and the coating materials affected the characteristics of capsules such as the content and the release feature of core material, the water proof and the swelling degree of capsules. With increasing the concentration of oil soluble surfactant, the released ratio decreased, become minimum and then, increased. The content could be increased by addition of modification materials. It was found that the released ratio was considerably depressed by ethyl laurate and ethyl palmitate as the modification materials and by oleic acid as the coating material and promoted by bees wax as the modification materials.

Keywords: L-cysteine containing capsules, tripalmitin, melting dispersion cooling method, release controlling, fatty acid esters.

I. Introduction

Many kinds of (micro) capsules have been prepared and applied in the various fields such as cosmetics, paintings, drugs, food, information recording materials, agricultural materials and so on [1-4].

The important functions of (micro) capsules are to protect the core material from environment and to controlly release the core materials [2,3]. These functions are largely dependent on the structure of (micro) capsules and the chemical and physicochemical properties of shell materials.

In general, the hydrophilic shell materials for the hydrophobic core materials and the hydrophobic shell materials for the hydrophilic core materials are used in order to protect the core materials from leaving into the continuous phase and to obtain the higher encapsulation efficiency. The hydrophilic solid powder as a fire retardant has been microencapsulated by the droplet coalescence method [5], the *in-situ* gelation method [6] and the interfacial reaction method [7]. These microencapsulation methods have been designed so as to increase the content by using the hydrophobic shell materials. B. Erdem, et al have microencapsulated TiO2 powder with the mini emulsion polymerization method, where the content of solid powder could be increased with the help of oil soluble surfactant having the larger hydrophilic groups [8-10].

Wang W, Zhon W have prepared the crystalline carbohydrate microcapsules containing soy sauce powder by the spray drying method [11]. The spray drying method can microencapsulate the hydrophilic solid powder with the hydrophilic shell materials. However, the microcapsules made by the hydrophilic shell material are easily swollen and rapidly release the core material. Especially, when the (micro) capsules will be applied to the limited fields such as food, drug and cosmetics, it is necessary to use the nontoxic edible shell materials and the materials suitable to the living body to prepare the (micro) capsules.

L-cystein is well known to be an essential amino acid and to have a few physiological effects such as anti-inflammation effect, anti-poison effect, whitening effect of skin and antiaging effect, but degenerate due to contact with water. Accordingly, it is worth encapsulating L-cystein with the hydrophobic shell materials.

In this experiment, it was tried to encapsulate L-cystein powder with tripalmitin with help of a few fatty acid esters as the modification materials and the coating materials in order to protect the core material from water attack and to controlly release the core material.

The purposes of this study are to try to encapsulate L-cystein powder with the melting dispersion cooling method by using tripalmitin as the shell material, to investigate how the modification materials and the coating materials affected the some characteristics of capsules such as the released ratio, the content of core materials and the swelling degree.

II. Experimental

a) Materials

Materials used to prepare the capsules containing L-cysteine were as follows.

Continuous phase : Distilled water

Stabilizer : methylcellulose(MC:Shinetsu Chemical Industry Co., Ltd.)

Core material : L-cysteine (Cys: Wako Pure Chemical Industry, Co., Ltd.)

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Shell material : Tripalmitin (TP: Kanto Chemical, Co., Ltd.)
Modification Materials : Ethyl Laurate (EL), Ethyl Palmitate (EP), Ethyl Myristate (EM), Ethyl Stearate (ES), Ethyl Oleate (EO), Bees wax (BW)
Coating materials : Oleic acid (OA), Triolein (TO), Ethyl laurate (EL), Ethyl Oleate (EO)
Oil soluble surfactant : Soy bean Lecithin (SBL)

The modification materials and the coating materials were from Kanto Chemical, Co., Ltd.

b) Preparation of capsules

The reactor was the separable flask with the effective volume of 300cm^3. The impeller used to form the (O/W) emulsion was the six bladed disc turbine with the diameter of 5.4cm which was set at one third of the liquid depth.

Figure 1 shows the flow chart for preparing the capsules. L-cysteine (Cys) of a given weight was added into Lecithin (SBL) and stirred to form the (S/O) dispersion. The (S/O) dispersion was added into the melted Tripalmitin (TP) and stirred for ten min to form the (S/(O+O')) dispersion. Next, the (S/(O+O')) dispersion was added into the continuous water phase dissolving methyl cellulose (MC) and stirred for ten min to form the (S/(O+O')/W) dispersion. The operation stated just above was performed at 74 ℃. After stirring the (S/(O+O')/W) dispersion to form the (S/(O+O')) droplets with the desired diameter for twenty min, the (S/(O+O')/W) dispersion was cooled down to 30℃ to solidify the Tripalmitin (TP) shell and then, the capsules containing L-cysteine (Cys) were prepared. In this fundamental operation, the modification agents were added in Tripalmitin (TP).

Furthermore, the capsules were coated with a few coating materials as follows.

The capsules of 0.2g were added into the bottle with the effective volume of 10cm^3 in which the melted coating materials of 50cm^3 were poured beforehand as shown in Figure 1. After soaking the capsules for a given time, the capsules were dried at room temperature. In the fundamental experiment stated above, the concentration of Lecithin (SBL), the kinds of modification materials and coating materials and the soaking time were changed. The experimental conditions were shown in Table 1.

Table 1 : Experimental conditions

Continuous water phase	
distilled water	290 cm^3
Methyl cellulose	0.29g (0.1 wt%)
Dispersed phase	
L-cysteine (core)	8.0 g
Tripalmitin (shell)	8.0 g
Soy bean Lecithin	2.0, 4.0, 6.0, 8.0 g
Preparation of dispersion	
Impeller speed	10 s^{-1}
Temperature	
Melting	74 ℃
Cooling	30 ℃
Modification materials:	0.8 g
Ethyl laurate, Ethyl stearate, Ethyl myristate, Ethyl oleate, Ethyl palmitate, Bees wax	
Coating materials: Oleic acid, Ethyl oleate, Triolein, Ethyl laurate	
Soaking time	24

c) Characterization

i. *Diameters of capsules*

The diameters of capsules were obtained directly from the photographs taken by the optical microscope. The mean diameters were the Sauter mean diameters.

ii. *Content of core material*

The content (Y) of core material encapsulated was defined as equation (1).

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$$Y = \frac{(weight\ of\ Cys\ encapsulated)}{weight\ of\ Cys\ in\ feed} \quad (1)$$

Here, the content of core material was obtained as follows.

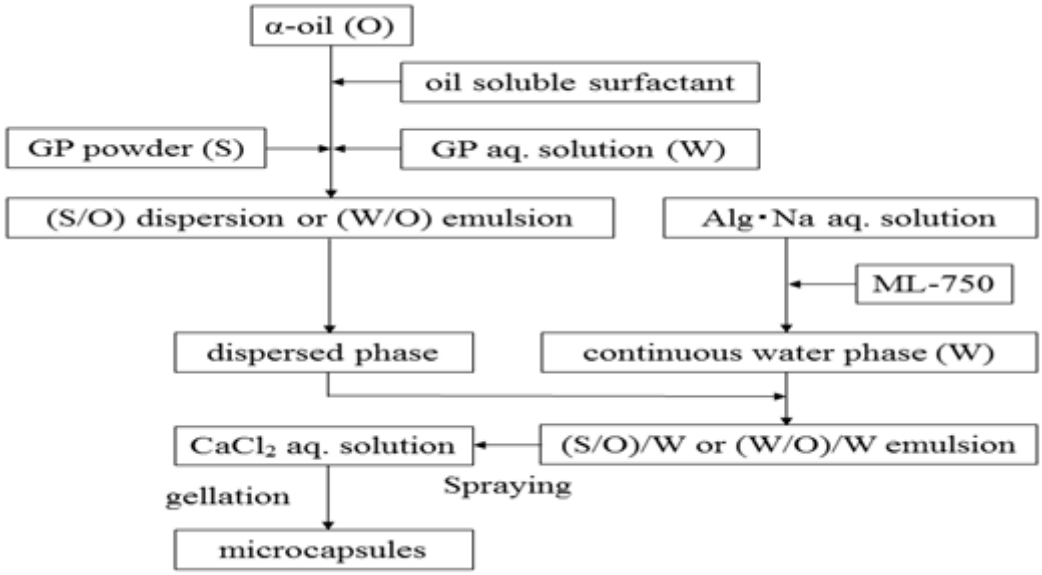

Figure 1 : Flow chart for preparing microcapsules

The capsules of 0.2g and distilled water of 10cm^3 were added into the beaker with the volume of 100cm^3. This beaker was kept in the refrigerator for 24h in order to swell the capsules by water. After breaking the capsules by the homogenizer and adding the distilled water of 100cm^3, ultrasonic irradiation to the capsule slurry was performed for twenty min in order to break the capsules and to dissolve out L-cysteine (Cys) perfectly. The aqueous solution dissolving L-cysteine (Cys) was filtered with the filter paper of 0.45μm, poured into the ultra filter vessel and then, filtered with the centrifugal separator.

The sample solution obtained by the procedure stated just above was sent to the high performance liquid chromatography (HPLC) and the amount of L-cysteine (Cys) was measured. The moving phase used in this measurement was prepared as follows. 0.58g of phosphoric acid of 85wt%, 0.342g of perchloric acid tetra-n-butylammonium and distilled water of 1000cm^3 were stirred. Then, pH of this aqueous solution was adjusted to pH 3.8 by adding 5N sodium hydride. The aqueous solution of 1000cm^3 thus adjusted was used as the moving phase.

Also, the colum used was Inertsil OD-3 (4.6ϕ × 150mm) (GL Science Ind. Ltd). In this measurement, temperature, the wave length and the liquid velocity were 40℃, UV 210nm, 0.7mol/min, respectively.

iii. *Observation of capsules*

The capsules were observed by optical microscope and scanning electron microscope (SEM: JSM-5800). In order to observe the inner structure of capsule, a capsule was cut into two pieces with the knife and was observed by scanning electron microscope.

iv. *Released ratio of core material*

Capsules of 0.2g were added in the beaker where distilled water of 100cm^3 was poured beforehand, and soaked for 24h at room temperature. Here, 5cm^3 of ampicillin sodium aqueous solution of 0.01vol% was dissolved in distilled water in order to prevent L-cysteine (Cys) from being consumed by microorganism.

Then, the aqueous solution was sampled out at the constant time intervals and the concentration of L-cysteine (Cys) dissolved was measured by HPLC after filtrating with filter paper of 0.45μm. Thus, the released ratio (R) was estimated by equation (2).

$$R(\%) = \frac{weight\ of\ Cys\ released}{weight\ of\ Cys\ encapsulated} \times 100 \quad (2)$$

v. *Swelling and break up of capsule*

After soaking the capsules into distilled water for 24h, the photographs of capsules were taken by digital camera. From these photographs, the swelling feature was observed and the number of capsules broken was counted.

vi. *Contact angle of water for composite shell film*

In order to obtain the informations about the capsules swollen by water, the composite shell film composed of Tripalmitin (TP) and the modification materials was prepared on the slide glass plate.

Then, a water droplet of 0.01cm^3 was formed on the composite shell film by microsyringe and taken the photograph by digital camera. From this photograph, the width(L) and height(H) of a water droplet were measured directly and the contact angle (θ) was estimated by equation (3).

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$$\theta = 2\tan^{-1}(2H/L) \quad (3)$$

III. Results and Discussion

a) Effect of concentration of SBL

Figure 2 shows the dependences of mean diameters (dp) of capsules and the content (Y) of L-cysteine (Cys) on the concentration of Lecithin (SBL) (C_{SL}). The mean diameters slightly increased from 2.0mm to 3.0mm with the concentration of SBL because of increase in viscosity of oil phase composed of Lecithin (SLB) and Tripalmitin (TP). Namely, the viscous force against the destructive force for an oil droplet become larger with the viscosity of oil phase [12,13]. As a result, the oil droplets become larger, because it is hard for an oil droplet to break up. On the other hand, the content rapidly increased with the concentration of SBL, become maximum at C_{SL}=0.75 and then, decreased at C_{SL}=1.0. Figure 3 shows the optical microscopic photographs (a) and the SEM photographs of surface (b) and the cross sections (c) of capsules prepared by changing the concentration of SBL. From these photographs, it was found that the surface of capsules was rough and the many tiny holes were in the matrix. These tiny holes may be caused by difference in crystal structures of Tripalmitin (TP) and Lecithin (SBL). Namely, Tripalmitin (TP) has the property of film formation, but Lecithin (SBL) is crystallogenic. Accordingly, many tinny holes may occur due to difference in phase separation and crystalline. The sudden decrease in the content at C_{SL}=1.0wt% as shown in Figure 2 may be due to these tinny holes. Namely, L-cysteine (Cys) may be dissolved by water permeating into the matrix through these tinny holes.

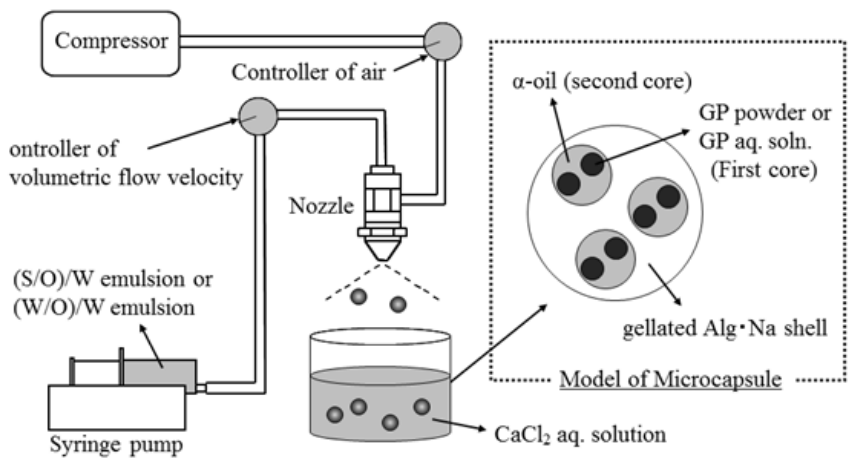

Figure 2 : Schematic diagram of experimental apparatus

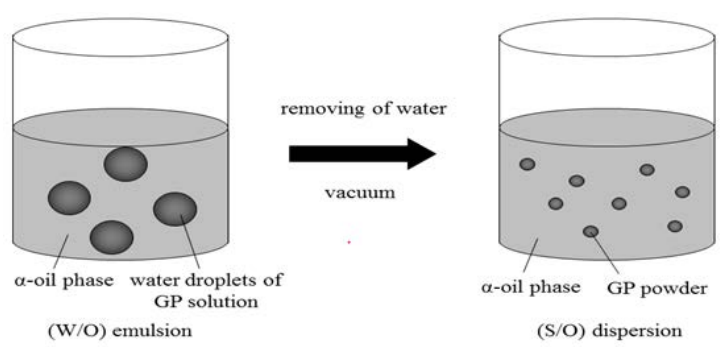

Figure 3 : Scheme for changing (W/O) emulsion to (S/O) dispersion

Figure 4 shows the transient features of released ratios for the capsules prepared by changing the concentration of SBL. With the concentration of SBL, the released ratio decreased, but increased at C_{SL}=1.0wt%. The decrease in the released ratio with the concentration of SBL may be due to the protection effect of Lecithin (SBL) against permeating of water. But, the increase in the released ratio at C_{SL}=1.0wt% is coincident with the lower content at C_{SL}=1.0wt% shown in Figure 2. From these results, it was found that the released ratio could be controlled by the concentration of SBL.

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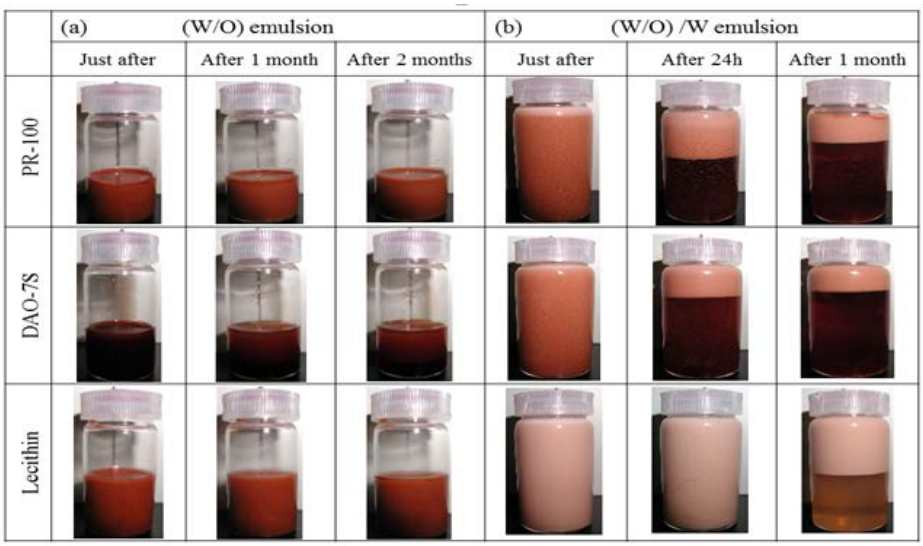

Figure 4 : Effect of oil surfactant species on stability of (W/O) emulsion and (W/O)/W emulsion

Figure 5 shows the optical microscopic photographs of capsules immersed in water. Just after (0h) immersion of capsules into water, any differences in the shape of capsules were not observed, but after 24h, the degrees of swelling increased with the concentration of SBL. However, the capsules broken were not observed irrespective of the concentration of SBL. The released ratios decreased in the region of the concentration from 0 to 0.75wt% within 150min as shown in Figure 4. However, after elapsing 24h, the capsules prepared by the concentration of SBL from 0~1.0wt% may be swollen by permeation of water.

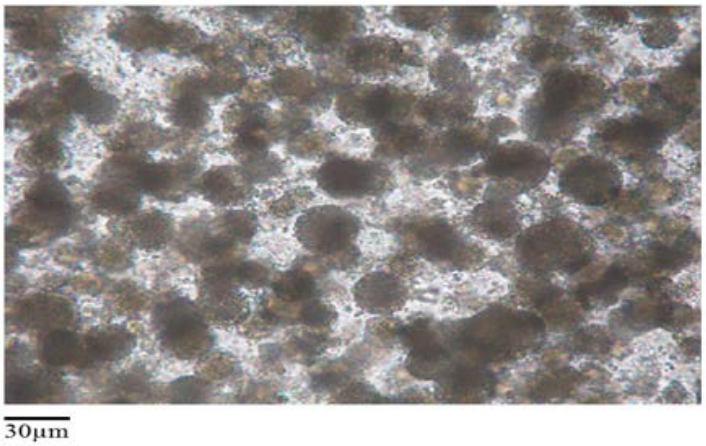

Figure 5 : Optical Microscopic Photographs of (W/O)/W Emulsion

b) Effect of modification materials

As the content, the swelling and the released ratio are strongly affected by permeation of water into the matrix of capsules, it may be necessary to give the hydrophobicity to the shell in order to prevent water from permeating. So, it was tried to modify the shell by adding the modification materials. For this, the effect of modification materials on the contact angle of water for the capsule shell was investigated. The contact angles of water for the shell were measured for the composite shell film.

Figure 6 shows the photographs of a water droplet on the composite shell film and the dependence of contact angle of a water droplet on the modification materials. It was found that the contact angles were not changed largely by adding the modification materials, but slightly increased by adding ethyl laurate (EL) (θ=119.8), ethyl myristate (EM) (θ=112.6) and ethyl stearate (ES) (θ=110.1).

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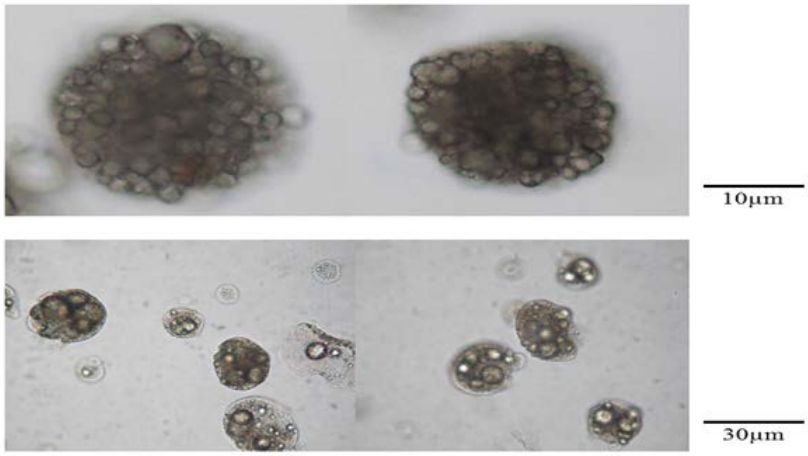

Figure 6 : Optical Microscopic Photographs of Microcapsules

Figure 7 shows the dependences of mean diameter (d_p) and content (Y) on the modification materials. The mean diameters were not changed largely by adding the modification materials, but the content could be increased by addition of ethyl laurate (EL) and ethyl stearate (ES) which have the larger contact angle. These results may be due to the fact that the modification materials increased the protective effect for water entering.

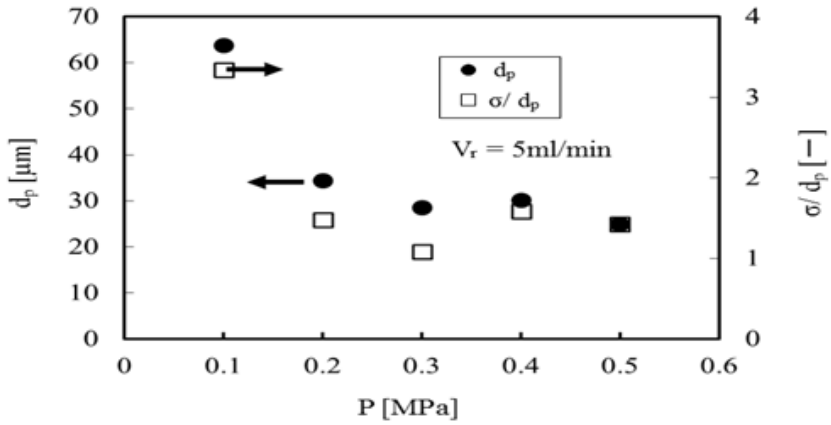

Figure 7 : Dependences of mean Diameter and Dispersion Degerr on Spraying Pressure

Figure 8 shows the photographs of capsule (a), the surface (b) and the inner structure (c) for the capsules prepared by adding the modification materials From these photographs, it was found that the surface of capsules become more smooth in comparison with the photographs in Figure 3 and the tinny holes in the capsule decreased.

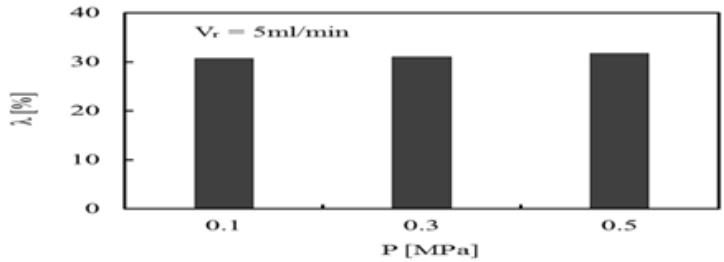

Figure 8 : Dapendences of Microencapsulation Efficiency of Spraying Pressure

Figure 9 shows the transient features of the released ratios measured for the capsules prepared by adding the modification materials. The released ratio was considerably decreased by addition of ethyl laurate (EL), ethyl palmitate (EP) and ethyl stearate (ES). Contrary to this, the released ratios were decreased by addition of ethyl myristate (EM) and ethyl oleate (EO) until elapsing 1h and increased by addition of Bees wax.

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Figure 10 shows the dependences of degree of swelling on the modification materials. Just after (t=0) immersion of capsules, the capsules were not changed irrespective of the kinds of modification materials, but after elapsing 24h, all the capsules swelled to the almost same degree and did not dissolved.

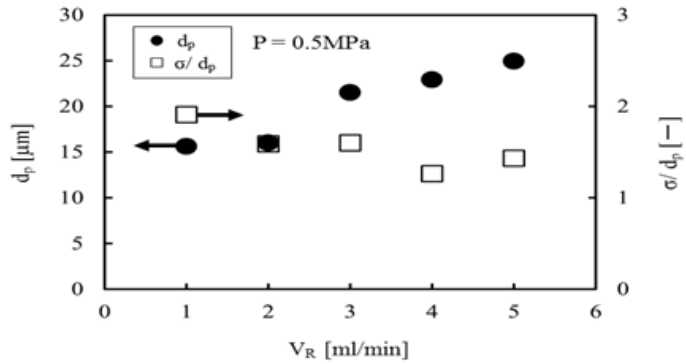

Figure 9 : Dependence of mean Diameter and Dispersion Degree on volumetric flow Velocity

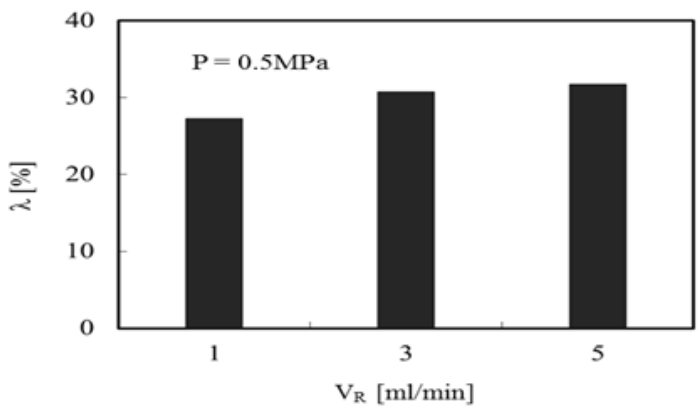

Figure 10 : Dependence of Microencapsulation Efficiency on volumetric flow Velocity

c) Effect of Coating materials on released ratio

In order to increase the releasing time of core material, the capsules were coated moreover by the coating materials. Namely, the capsules have the dual shell film.

Figure 11 shows the transient features of the released ratios for the capsules prepared by being immersed in the coating materials for 3days and 10days. The capsules immersed for 3days show that the released ratios are largely decreased, especially the effect of coating by oleic acid (OA) is considerably.

Furthermore, the capsules immersed for 10days show the extreme decrease in the released ratio, especially the effect of coating by oleic acid (OA) is considerably.

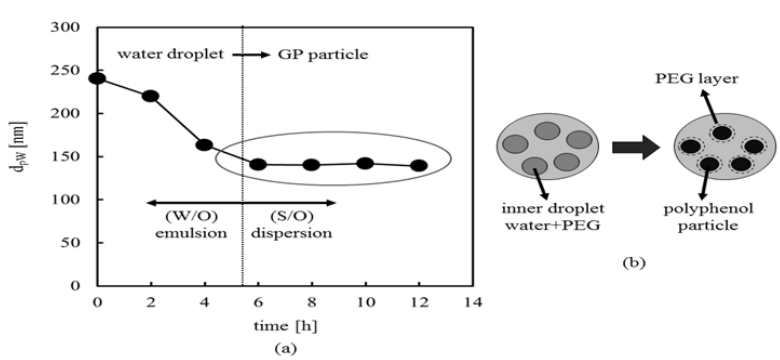

Figure 11 : Transient Inner water Droplet Diameters

Figure 12 shows the photographs of capsules immersed in water. The capsules coated with the coating materials except triolein (TO) were not practically swollen. These results show that the released ratios of core materials can be controlled for a long period by the coating materials.

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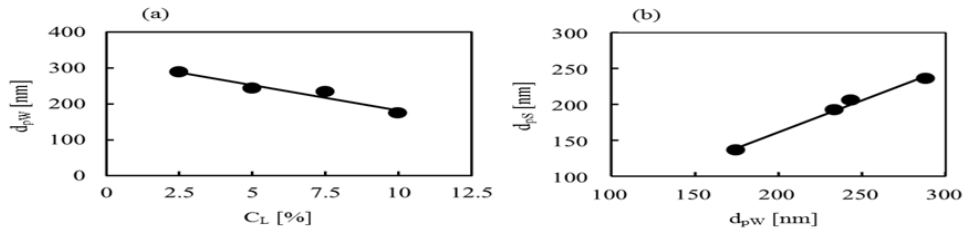

Figure 12 : Dependence of water Droplet Diameters on surfactant concentration and Dependence of GP Powder Particle Diameters on water Droplet Diameters

IV. Conclusion

L-cysteine powder was tried to encapsulate with tripalmitin as the shell material and the effects of modification materials and the coating materials on the characteristics of capsules were investigated.

The following valuable results were obtained

1. L-cysteine powder could be encapsulated with tripalmitin by using the melting dispersion cooling method.
2. The released ratio of L-cysteine could be controlled by addition of modification agents. Especially, ethyl laurate and ethyl palmitate could decrease the released ratio.
3. The content of core material could be increased by addition of modification agents.
4. The release ratio of L-cysteine could be largely decreased by coating the capsules with the coating materials. Especially, oleic acid could be considerably decreased the released ratio.
5. The time span for releasing L-cysteine could be controlled over the wide range by adding the modification materials and by coating with the coating materials.

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Comparative Study of Immunohistochemical, Hematoxylin & Eosin Staining and its Diagnostic Importance in Hirschsprung's Disease

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Abstract- Aim: A comparative study of immunohistochemical, hematoxylin & eosin staining and its diagnostic importance in Hirschsprung's disease.

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Results: In our study of 357 cases, 223 are male children and 74 are female children (Male: Female ratio-3:1). Short segment was the most commonly occurring type constituting 229 cases (64%), while long segment was 77 cases (21.5%). The less common is the total colonic aganglionosis constituting 21 cases (5.8%).

Conclusions: Cathepsin D is equally good like Acetyl cholinesterase and can be used as a reliable immune-histo chemical stain in detecting immature ganglion cells..

Keywords: hirschsprung's disease, immunehistochemi-cal stain, H and E stain.

GJMR-C Classification : NLMC Code: WI 528

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Lakshmi Vasavi.H [α], Inamdar.S.S [σ] & Uma.T [ρ]

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Keywords: hirschsprung's disease, immunehistochemical stain, H and E stain.

I. Introduction

Harald *H*irschsprung first described in 1888 two unrelated boys who died from chronic severe constipation with abdominal distension resulting in congenital megacolon.[1] Hirschsprung's disease (HD) is defined as the absence of ganglion cells in submucosal (Meissner's) and myenteric (Aurbach's) plexuses in distal bowel extending proximally from internal anal sphincter for variable distances that result in functional obstruction caused by dysmotility of the diseased segment.[2] It is one of the most common diseases in the field of pediatric surgery. Occurrence of the disease is 1 in 5000 live births. 70-80 percent of them are boys. Based on the age of diagnosis, the most cases of Hirschsprung's disease are diagnosed in neonatal period and the rest are discovered uptil 2 years of age. It is believed to result from the failure of ganglion cells to migrate caudally during the embryonic life. The loss of ganglion cells extends for a variable distance above the anorectal junction. The classical Hirschsprung's disease was found restricted to rectosigmoid junction in 75% of cases; long segment disease in 15% of cases, ultra short segment disease in 5% of cases and variable length was found in 5% of cases.[3] The aganglionic bowel in Hirschsprung's disease was diagnosed using HSCR in most of the newborn cases owing to intestinal obstruction with the following features are failure to pass meconium within the first 48 hours of life, vomiting, abdominal distension lacks the normal motility, functional obstruction that leads to neonatal enterocolitis.[4]

The diagnostic accuracy of various modalities for Hirschsprung's disease are radiology 60% (Barium enema) manometry 90%, biopsy 95% and immuno-histochemistry has 99% accuracy.[5] Present our study is to evaluate the diagnostic difficulties in identifying ganglion cells and to compare the utility of seromuscular biopsy over sub mucosal biopsy.

II. Material and Methods

This prospective study was carried out at Niloufer hospital, Hyderabad for a period of 6 years (from January 2000 to December 2005). The total number of surgical specimens and biopsies received at pathology Department of niloufer hospital, Hyderabad for 6 year period were 3844 out of which 357 cases were Hirschsprung's disease and rest 153 cases are other causes of constipation in pediatric age group [Table 1]. The surgical specimen's, colorectal specimens, appendicectomy, myectomy, biopsies at various levels of intestine were taken. The cases that presented with various causes of chronic constipation and intestinal obstruction such as Hirschsprung's disease, meconium ileus, ileal atresia, intestinal neuronal dysplasia and hypoganglionosis were examined by surgical biopsies and specimens [Table 2]. The study of 510 patients comprised colorectal, appendicectomy biopsies and myectomy specimens at various levels. The study included both ganglionic and aganglionic segments of intestine. The specimens were fixed in 10% formalin solution. In the laboratory, after preparing sections of

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paraffin embedded tissues, H and E staining slides were compared with Cathepsin D. Cathepsin D is a specific, sensitive marker that detects immature ganglion cells. Acetylcholine esterase is equally specific and sensitive, but neuron specific enolase (NSE) is a histochemical and IHC method, it will not help the detection of immature ganglion cells.

III. Results

Based on the age of diagnosis, most cases of Hirschsprung's disease are diagnosed in neonatal period and the rest are diagnosed until 2 years of age [Graph 1]. In our study of 357 cases, 223 are male children and 74 are female children (Male: Female ratio-3:1). Short segment was the most commonly occurring type constituting 229 cases (64%). The less common is the total colonic aganglionosis constituting 21 cases (5.8%); while long segment was 77 cases (21.5%) [Graph 2].

There were 20 cases of Hirschsprung's disease among the 96 subjects, 15 cases showed a positive pattern – A. In 13 of these patients, the fresh frozen, cryostat cut, and H & E stained sections showed the absence of neurons and the presence of hypertrophic nerve bundles in the submucosa [Table 3]. The H & E stain pointed to the diagnosis of Hirschsprung's disease in five other cases when the AChE pattern was other than pattern-A. The full thickened biopsies from the aganglion areas at the time of colostomy confirmed the diagnosis in all the 20 cases.

In Immuno-histochemistry (Cathepsin D) stains both immature and mature ganglion cells. Nerve fibers are not stained. Intense granular cytoplasmic staining is produced. This forms a collarets around the nucleus [Figure 5]

Table 1 : Clinical Comparison between Idiopathic constipation and Hirschsprung's disease

Signs, Symptoms and Diagnostic Studies	Idiopathic Constipation	Hirschsprung's disease
1. Soiling	Common	Unusual
2. Still in ampulla	Common	Unusual
3. Obstructive symptoms	Rare	Common
4. Stool retentive behavior	Common	Rare
5. Enterocolitis	Never	Possible
6. Anorectal examination findings	Dilated ampulla	Narrow
7. Contrast enema findings	Dilated ampulla	Narrowed distal segment

Table 2 : Hirschsprung's disease and other Causes of Constipation in Pediatric Age Group at Niloufer Hospital (2000-2005)

Disease	Total No. of Cases	% of Cases
Hirschsprung's disease	357	70%
Meconium Ileus	11	2.3%
Intestinal Atresia	21	4.1%
Intestinal neuronal Dysplasia	02	0.4%
Hypoganglionosis	10	2%
Normal	93	18.2%
Inadequate Biopsies	16	3.4%
Total	510	100%

Table 3 : Staining Results in Patients with and without Hirschsprung's disease

AChE pattern	Fresh frozen, cryostat cut, H & E stained section Results			
	With Hirschsprung's disease		Without Hirschsprung's disease	
	Neurons Absent	Hypertrophic Nerves present	Neurons Absent	Hypertrophic Nerves present
Pattern A (n=15)	13	13	0	0
Pattern B (n=3)	3	3	7	0
Equivocal (n=2)	2	2	5	0
Negative (n=0)	0	0	50	0

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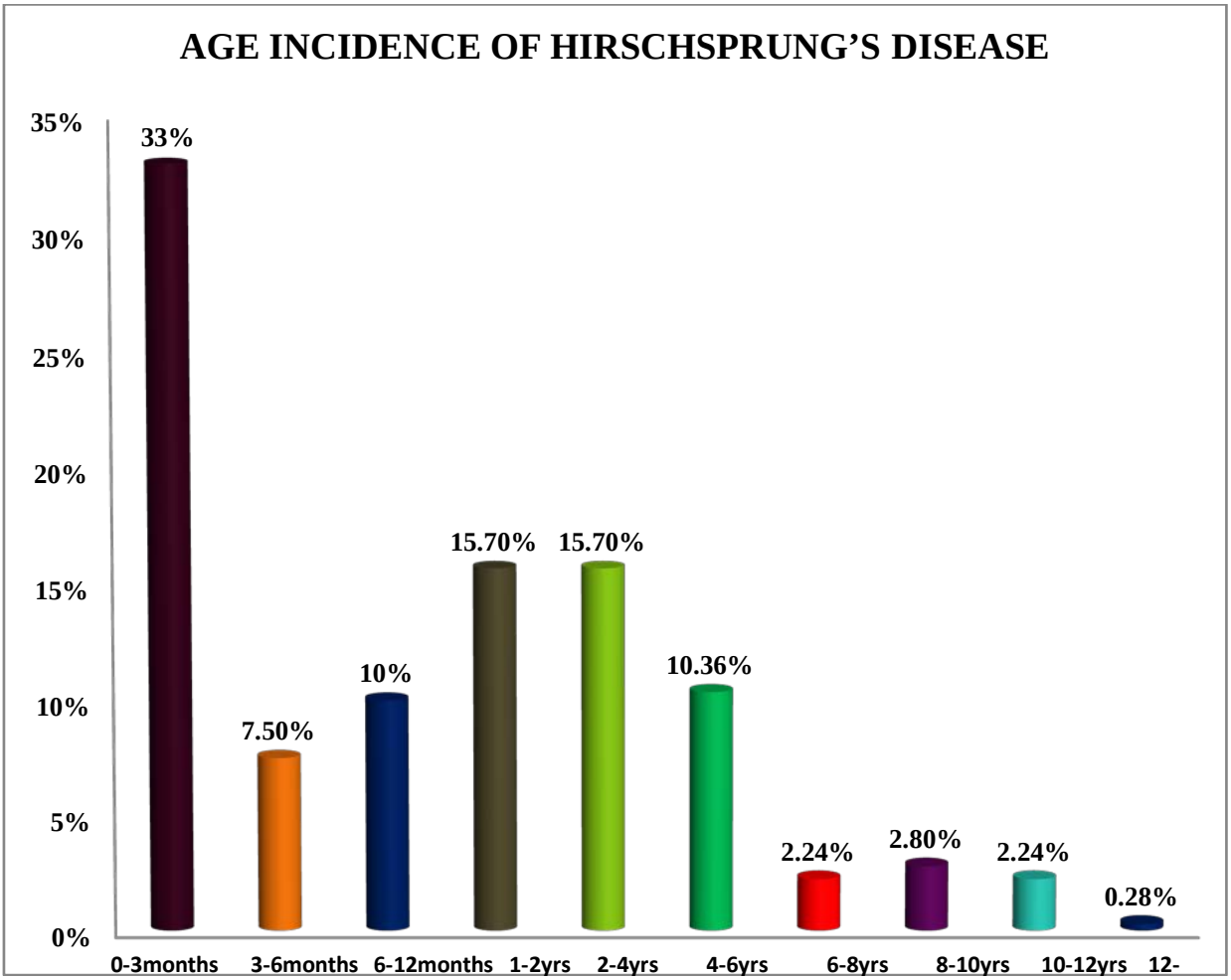
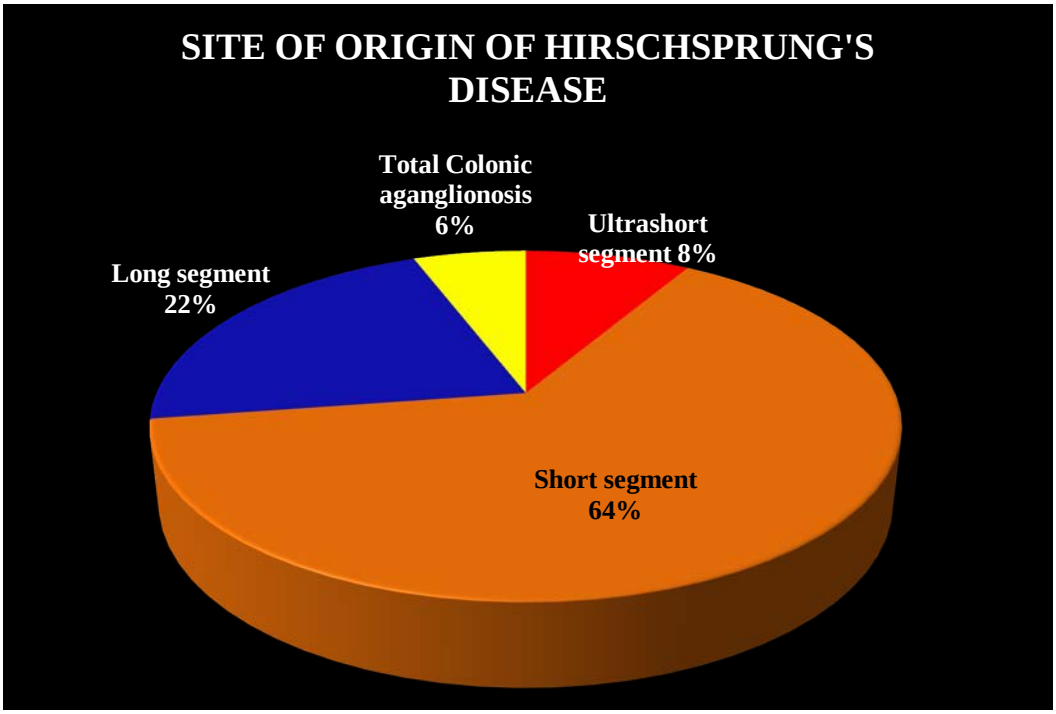


Graph 1



Graph 2

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IV. Discussion

Study of Hirschsprung's disease in pediatric age group was undertaken to observe the age and sex incidence, to study the various types of Hirschsprung's disease, the utility of seromuscular biopsy over sub mucosal biopsy and identify the diagnostic difficulties in detecting immature ganglion cells especially in total colonic aganglionosis. Detection of ganglion cells in H and E sections can be a difficult process for the pathologist.[6] The maturation of ganglion cells is incomplete at the time of birth, especially in the sub mucosal area.[7] Immature ganglion cells may be unipolar or bipolar and can be mistaken for stromal cells.[7] Sub mucosal ganglion cells are smaller than myenteric plexus ganglion cells,[8] and pathologists have to prepare between 50 to 400 sections of H and E stained slides to find ganglion cells.[9] On the other hand, although AChE staining is the chosen technique for some pathologists[10] its diagnosis needs experience and its interpretation is difficult in some instances.[11] One of the problems is the interference of red blood cell (RBC) is acetyl cholinesterase due to hemorrhage in lamina propria.[6] Also, false positive[9] and false negative [6] reactions were reported using this staining technique. Technical difficulties and storage problem of reagents is also reported. [10, 12, 13, 14]

In our study, short segment Hirschsprung's disease is the most common type involving 64.5% cases; lowest incidence is occupied by total colonic aganglionosis i.e., 6% [Graph 2]. In our study, almost 1/3rd (33%) of cases were established by the first 3 months of life, only 17% by the first year, from 1-6yrs, they are almost 40%. Beyond 6yrs i.e., 6-14 yrs is only 8% are reported [Graph 1]. The histochemical technique must be affordable with specificity and sensitivity for the detection of ganglion cells. In our study, cathepsin D was performed on several formalin fixed paraffin embedded blocks. It involved both aganglionic [Figure 4] and ganglionic segments of intestine.

Cathepsin D and AChE are the only stains to detect immature ganglion cells [Figure 6]. In total colonic aganglionosis this is the only stain helps for a definite diagnosis. Cathepsin D is the only stain which stains immature and mature ganglion cells along with AChE but in cases of total colonic aganglionosis [Figure 4], this panel can detect smaller or immature ganglion cells and also small cytoplasmic portions of those cells [Figure 5]. Hence, the sensitivity and specificity is increased with false negative and decreased with false positive results.

V. Conclusion

Comparing the results of Cathepsin D with Acetyl cholinesterase, Cathepsin D was found to be equally good like acetyl cholinesterase and useful as a reliable immune-histochemical stain in detecting immature and mature ganglion cells. Following colostomy in patients with Hirschsprung's disease, few of them are prone to develop neonatal enterocolitis and perforation. This enterocolitis may be due to improper level colostomy. So to detect this it is essential that the presence of ganglion cells should be looked for in the colostomy site biopsy which helps in differentiating neonatal enterocolitis due to improper colostomy from other etiologies.

Therefore it is emphasized that correct level for colostomy surgery is to be checked with biopsy of the colostomy site and this biopsy must also be subjected to immuno-histochemistry.

Figure 1 : Classical segment Hirschprung's disease (40X) – Hypertrophied nerve bundles

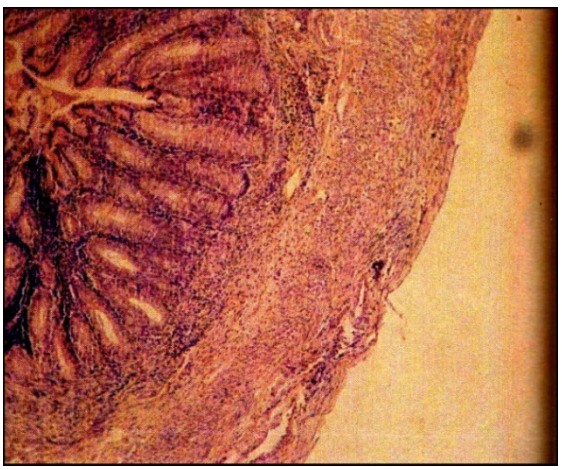

Figure 2 : Classical segment Hirschprung's disease (10X) – Hypertrophied nerve

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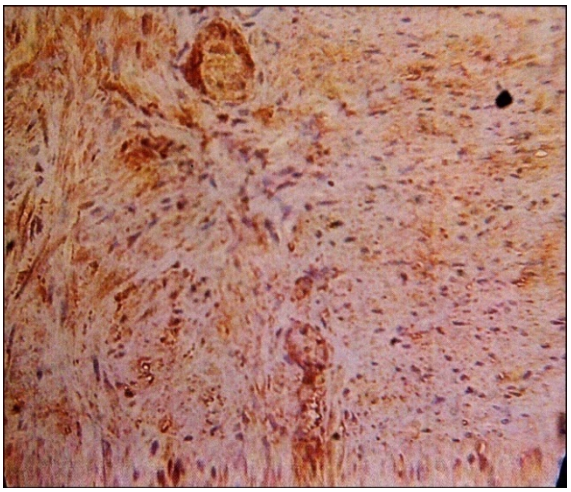

Figure 3 : Cathepsin D positive – Myenteric plexus

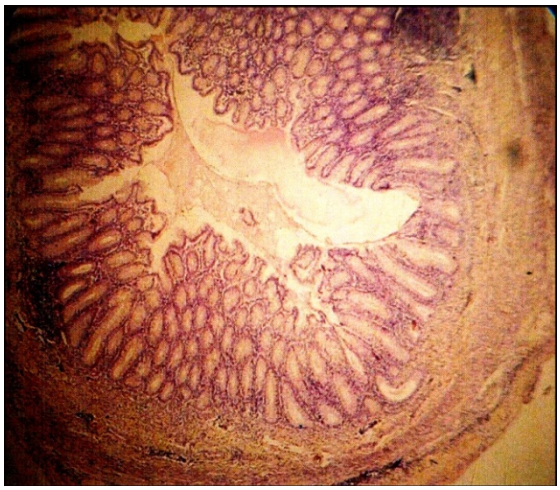

Figure 4 : Low power view (10X) - Appendix in a case of suspected total colonic

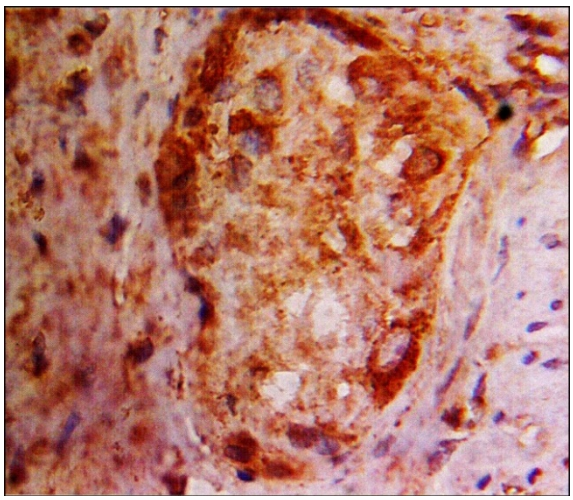

Figure 5 : Cathepsin D positive – Intense granular cytoplasmic reactivity with collarette around nucleus

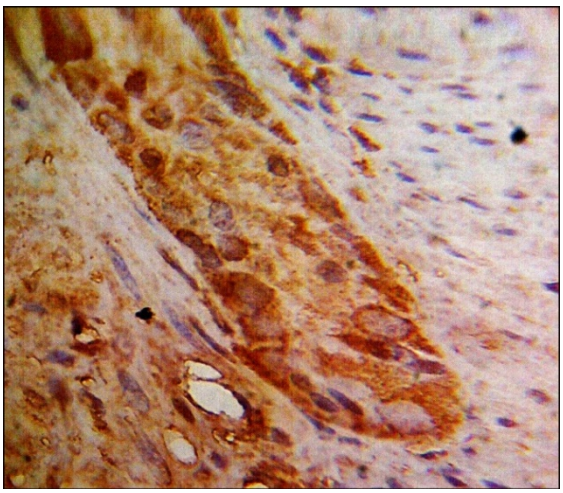

Figure 6 : Typical ganglion cell complex – with Cathepsin D positivity

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Correlation between the use of Antimicrobials and the Occurrence of Antimicrobial Resistant Bacteria in Poultry and Pig Farms

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Abstract- Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. A survey was carried out to evaluate the relationship between the use of antimicrobials in animal production and the occurrence of antimicrobial resistant organisms. The survey was conducted between November, 2012 to May 2013 using structured questionnaires. Responses to the questionnaires were analyzed using linear regression and correlation variables. Results showed that correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria were both positive and negative on one hand and significant and non-significant on the other hand at 0.01 and 0.05 in both poultry and pig farms. Escherichia coli isolates had a negative (-0.20) non significant ($P>0.050$) correlation with increase in dosage of antimicrobial given. Negative, non-significant ($P>0.05$) correlations were found between dosage of antimicrobials given and number of Enterococcus isolates (-0.19). In Table 2, the correlations between the variables were almost positive except between dosage of antimicrobials given and number of Enterococcus isolates where there was no correlation.

Keywords: antimicrobial usage, occurrence, resistant bacteria, poultry and pig farms.

GJMR-C Classification : NLMC Code: QW 4

CORRELATIONBETWEENTHEUSEOFANTIMICROBIALSANDTHEOCCURRENCEOFANTIMICROBIALRESISTANTBACTERIAINPOULTRYANDPIGFARMS

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Correlation between the use of Antimicrobials and the Occurrence of Antimicrobial Resistant Bacteria in Poultry and Pig Farms

N. Amaechi

Abstract- Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. A survey was carried out to evaluate the relationship between the use of antimicrobials in animal production and the occurrence of antimicrobial resistant organisms. The survey was conducted between November, 2012 to May 2013 using structured questionnaires. Responses to the questionnaires were analyzed using linear regression and correlation variables. Results showed that correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria were both positive and negative on one hand and significant and non-significant on the other hand at 0.01 and 0.05 in both poultry and pig farms. *Escherichia coli* isolates had a negative (-0.20) non significant (P>0.050) correlation with increase in dosage of antimicrobial given. Negative, non-significant (P>0.05) correlations were found between dosage of antimicrobials given and number of Enterococcus isolates (-0.19). In Table 2, the correlations between the variables were almost positive except between dosage of antimicrobials given and number of Enterococcus isolates where there was no correlation. Results from linear showed that farm size and level of education were significant at 5% and 10% in poultry and pig farms respectively. The results of this study suggest that the amounts and patterns of antimicrobials used in food animals is the major determinant for the propagation of resistant bacteria in the animal reservoir. However, further studies are needed for other determinants that may play a part in the propagation of resistant bacteria in animal reservoir.

Keywords: antimicrobial usage, occurrence, resistant bacteria, poultry and pig farms.

I. Introduction

There has been massive use of antimicrobials in animal husbandry. The most abundant use of antimicrobials worldwide is in livestock; they are typically distributed in animal feed and water for purposes such as disease prevention and growth (Silbergeld *et al.*, 2008). Debates have arisen surrounding the extent of the impact of these antimicrobials, particularly antimicrobial growth promoters, on human antimicrobial resistance. Although some sources believe that there remains a lack of knowledge on which antimicrobial use generates the most risk to humans (Landers *et al.*, 2012).

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The use of antibiotics has been linked to the rise of resistance in every drug and species where it has been studied, including humans and livestock. The use of antimicrobials in various forms in widespread throughout animal industry. The practice of using antimicrobials for growth stimulation is problematic as it is the longest use of antimicrobials worldwide (Silbergeld *et al.*, 2008). Its sub therapeutic use results in bacteria resistance (Silbergeld *et al*; 2008) and every important class of antimicrobial are being used in this way, making every class less effective (Sillbergeld *et al.*, 2008).

There has been a study on whether there was a connection between resistance and the practice of feeding a drug related to vancomycin to animals as a growth stimulant (Landers *et al.*, 2012). Vancomycin-resistant enterococci can spread from animals to humans (Wegner, 2003) CC 398 is a methicillin-resistant *Staphylococcus aureus* which was produced by the use of antibiotics in livestock production (Peter *et al.*, 2008). The appearance of carbepenem resistant entero-bacteriaceae has been attributed in part to antibiotic in livestock (Carlet *et al.*, 2012). The overuse of fluoroquinolone and other antibiotics fuels antimicrobial resistance in bacteria, which can inhibit the treatment of antimicrobial-resistant infections (Nauhauser, *et al.*, 2003). Widespread use of fluoroquinolones as a first-line antibiotic has led to decreased antimicrobial sensitivity, with negative implications for serious bacterial infections such as those associated with cystic fibrosis, where quinolones are among the few viable antibiotics (Ziganshina and Squire, 2008).

Although microbial resistance results primarily as a consequence of selection pressure placed on a susceptible microbes by the use of therapeutic agents, a variety of social and administrative factors also contribute to the emergence and spread of resistance. The aforementioned factors necessitated the need to carry out this study.

II. Materials and Methods

a) Poultry and Pig Farms

A total of 70 poultry and 50 pig farms were randomly selected from the 17 local government areas of Abia State, Nigeria were selected. The poultry and pig

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farms that participated in this study were managed intensively and were classified as large and commercial poultry and pig farms.

b) Survey Questionnaire

A survey instrument (questionnaire) on antimicrobial usage was developed for collecting information on antimicrobial usage. The questionnaires were administered by the author to the manager or the owners of each farm. The questionnaire sought information like dosage of antimicrobials given, frequency of antimicrobial use, duration of administration, who makes prescription etc as well as personnel data.

c) Statistical Analysis

Answers to the questionnaires were analyzed using linear regression where X is the independent variables and Y is the dependent variables. Correlation analysis was done to determine the relationship between antimicrobial usage and the occurrence of antimicrobial resistant bacteria in poultry and pig farms at 0.01 and 0.05 levels.

III. Results

A significant reason for high selection pressure in the face of modest antimicrobial expenditure is inappropriate antimicrobial use. Table 1 shows the correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria in pig farms. The correlation among some variables was both positive and negative on one hand, and significant and non-significant on the other hand. For instance, the correlation between dosage of antimicrobial given (X_1) and frequency of antimicrobial use (X_2) was positive (0.46) and significant ($P<0.05$). This implies that the dosage and frequency of antimicrobial have positive association such that increase in the frequency of use will lead to increase in the dosage of antimicrobials. Dosage of antimicrobial given and number of *Escherichia coli* isolates had a negative (-0.20) non-significant ($P>0.05$) correlation, implies that *E.coli* isolates will decrease with increase in dosage of antimicrobials.

Table 1 : Correlation between the use of Antimicrobials and the Occurrence of Antimicrobial Resistant Bacteria in Pig Farms

	X1	X2	X3	X4	X5	X6
X1	1					
X2	0.46**	1				
X3	0.28*	0.36**	1			
X4	0.30*	0.23	0.16	1		
X5	-0.20	-0.31*	-0.27*	0.10	1	
X6	-0.01	-0.19	-0.15	0.15	0.93	1

*** = correlation is significant at 0.01 levels*
**= correlation is significant at 0.05 levels*
X1= Dosage of antibiotics given
X2 = Frequency of antimicrobial use
X3 = number of animals in the flock that received antimicrobials
X4 = Completion of antimicrobial treatment
X5= Number of E. coli isolates
X6 = Number of Enterococcus isolates

Generally, positive and significant ($P<0.05$) correlations existed between each of dosage of antimicrobial given and frequency of antimicrobial use, dosage of antimicrobial given and number of animals in the flock that received antimicrobial; dosage of antimicrobial given and completion of antimicrobial treatment and frequency of antimicrobial use and number of animals in the flock that received antimicrobial with correlation coefficient of 0.46, 0.28, 0.30 and 0.36 respectively.

Correlation between each of frequency of use of antimicrobials and completion of antimicrobial treatment; number of animals in the flock that received antimicrobials and completion of antimicrobial treatment; completion of antimicrobial treatment and number of *E. coli* isolates; completion of antimicrobial treatment and number of Enterococcus; and number of *E.coli* isolates and number of Enterococcus isolates were positive and non significant ($P>0.05$) with respective correlation coefficients of 0.23, 0.16, 0.10, 0.15 and 0.93 respectively.

Negative significant ($P<0.05$) correlations existed between frequency of use of antimicrobials and number of *E.coli* isolates (-0.31) and between number of animals in the flock that received antimicrobials and number of *E.coli* isolates (-0.27), while negative non-significant ($P>0.05$) correlations were found between dosage of antimicrobials given and number of *E.coli* isolates (-0.20) dosage of antimicrobial given apnd number of Enterococcus isolates (-0.01); frequency of antimicrobial use and number of Enterococcus isolates (-0.19); and number of Enterococcus isolates and number of animals in the flock that received antimicrobials (-0.15).

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Table 2 : Correlation between the use of Antimicrobials and The Occurrence of Antimicrobial Resistant Bacteria in Poultry Farms

	X1	X2	X3	X4	X5	X6
X1	1					
X2	0.12	1				
X3	0.30*	0.58**	1			
X4	0.27*	0.32*	0.36**	1		
X5	0.39**	0.24*	0.29*	0.41**	1	
X6	0.00	0.26*	0.26*	0.17	0.50*	1

*** = correlation is significant at 0.01 levels*
**= correlation is significant at 0.05 levels*
X1= Dosage of antibiotics given
X2 = Frequency of antimicrobial use
X3 = number of animals in the flock that received antimicrobials
X4 = Completion of antimicrobial treatment
X5= Number of E. coli isolates
X6 = Number of Enterococcus isolates

Table 2 above showed the correlation between the use of antimicrobials and the occurrence of antimicrobial resistance bacteria in poultry farms. The correlations between the variables were almost positive except between dosage of antimicrobial given and number of Enterococcus isolates where there was no correlation. Dosage of antimicrobial given and frequency of antimicrobial use; completion of antimicrobial treatment and number of Enterococcus isolates each had positive non-significant ($P>0.05$) correlation with coefficients of 0.12 and 0.17 respectively. Other positive correlations were all significant ($P<0.05$).

Table 3 shows the regression of dependent variables, the most common antimicrobial use (Y1) and frequency of use (Y2) on the dependent variables using four functional forms- linear, semi-log, double log and exponential in poultry farms. The values outside the parenthesis between Y1 and Y2 and each of the X's are the regression coefficients, while those in the parenthesis are the t-statistics. For instance, the linear regression coefficient between Y1 and each of X1, X2 and X3 are 0.46, 0.11 and 0.43 respectively and that of Y2 are -0.02, 0..17 and -0.12 respectively.

Table 3 : Regression Analysis in Poultry Farms Regression Functions

Explanatory variable	Linear		Semi-log		Double log		Exponential	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Constant	-2.10 (-1.19)*	1.36 (1.45)*	-0.27 (-0.31)*	1.50 (3.18)**	-0.50 (-1.18)	0.37 (1.33)*	-1.41 (-1.66)*	0.37 (0.67)
X1	0.46 (2.75)**	-0.02 (-0.22)	1.14 (2.65)**	-0.12 (-0.53)	0.57 (2.76)	-0.10 (-0.76)	0.23 (2.88)**	-0.23 (-0.44)
X2	0.11 (-0.59)	0.17 (1.71)	-0.29 (-0.81)*	0.41 (2.16)**	-0.20 (-1.15)*	0.25 (2.21)**	-0.9 (-0.98)	0.10 (1.70)*
X3	0.43 (1.87)*	-0.12 (-0.15)	0.78 (1.89)*	-0.17 (-0.78)*	0.37 (1.88)*	-0.11 (-0.86)	0.20 (1.79)*	-0.08 (-1.13)*
X4	0.34 (1.37)*	-0.52 (-1.15)	0.51 (1.14)*	-0.31 (-1.33)*	0.25 (1.19)*	-0.15 (-1.05)*	0.17 (1.39)*	-0.08 (-1.00)*
X5	-0.06 (-0.27)	0.35 (2.90)**	-0.18 (-0.40)	0.56 (2.41)**	-0.12 (-0.59)	0.37 (2.62)**	0.05 (-0.44)	0.21 (2.90)**
X6	-0.01 (-0.05)	-0.05 (-0.50)	-0.10 (-0.31)	-0.10 (-0.58)	-0.05 (-0.31)	-0.06 (-0.67)	0.00 (0.01)	-0.04 (-0.66)
X7	-0.001 (-0.004)	0.09 (0.79)*	0.14 (0.20)	0.29 (0.77)*	0.13 (0.38)	0.16 (0.71)	0.02 (0.18)	0.08 (1.24)*
X8	0.64 (1.55)	1.36 (0.63)	0.92 (1.51)	0.16 (0.49)	0.46 (1.59)*	0.07 (0.37)	0.33 (1.64)*	0.06 (0.49)
R^2	0.24	0.36	0.24	0.36	0.11	0.38	0.27	0.40
Error term	1.81	1.89	1.81	1.92	1.80	1.87	1.83	1.81
F statistics	1.35*	2.46**	1.35*	2.41**	1.68*	2.62**	1.60*	2.86**

*** = significant at 5%*
** = significant at 10%*
R^2 = coefficient of determination

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Values in parenthesis are + statistics of individual X variables
X = Independent variable(X1 =level of education; X2 = farm size, X3 = Reason for antimicrobial use, X4 = Duration of administration, X5 = who makes the prescription, X6= Reason for treatment using antimicrobial, X7= Frequency of consulting a veterinarian, X8= Availability of veterinarian when needed.
Y = dependent variable (Y1 = the most common antimicrobial use, Y2 = frequency of use)

The linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education led to 0.46 increases in the most common antimicrobial use, and this was significant at 5%. Thus level of education is a determinant factor in the use of antimicrobials. Increase in the farm size in poultry farming will lead to increase use of a particular antimicrobial due to increased assessment of market information. Thus social factor may play an important role in the success or otherwise of poultry farming.

Table 3 also showed that the coefficient of multiple determinant (R^2) for Y1 and Y2 in linear, semi-log,, double log and exponential regression functions were 0.24,0.36, 0.24, 0.36, 0.11, 0.38, 0.27 and 0.40 respectively. The R^2 indicates the total variation in Y (dependent variable) that is caused by X's (the independent variables). The values of R^2 were greatly low, below 50%, the highest being 0.40, between frequency use and the independent variables. This indicates that about 40% of the total variation in the most common antimicrobial use was caused by the combined effect of the X1-X2.

Table 4 showed the regression of dependent variables, the most common antimicrobial use (Y1) and frequency of use (Y2) on the dependent variables using four functional forms- linear, semi-log, double log and exponential in pig farms. The values outside the parenthesis between Y1 and Y2 and each of the X's are the regression coefficients (bs), while those in the parenthesis are the t-statistics. Taking X1, X2 and X3 as example, the linear regression coefficients between Y1 and each of X1, X2 and X3 are 0.08, 0.22 and -0.19 respectively and that of Y2 are 0.17, 0.69 and -0.03 respectively.

The semi-log regression coefficients of these variables are- 0.04, 0.48 and 0.22 for Y1and 0.27, 1.38 and 0.17 for Y2 respectively. The double log regression coefficients of these variables are -0.03, 0.24 and -0.14 forY1 and 0.17, 0.61 and -0.06 for Y2 respectively. Similar results for the exponential regression are 0.04, 0.11 and-0.11 for Y1 and 0.09, 0.31 and -0.04 for Y2 respectively. The linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education led to 0.08 increases in the most common antimicrobial used, and this was not significant. Similarly, as farm size increased, frequency of use of antimicrobial increased in pig farms and this was significant at 10%.

In Table4, the coefficient of multiple determinants (R^2) for Y1 and Y2 in linear, semi-log, double log and exponential regression functions were 0.15, 0.34, 0.20, 0.31, 0.20, 0.34, 0.14 and 0.37 respectively. The values of R^2 were generally smaller than 50%, the highest being 0.37, between frequency of use and the independent variables. This indicates that about 37% of the total variation in the most common antimicrobial used was caused by the combined effect of the VII-VIII.

Table 4 : Regression Analysis in Pig Farms Regression Functions

Explanatory variable	Linear		Semi-log		Double log		Exponential	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Constant	2.27 (1.86)*	0.20 (0.16)	2.63 (3.67)***	0.82 (1.12)*	0.95 (2.45)**	0.17 (0.58)	0.75 (0.13)*	-0.03 (-0.06)
X1	0.08 (0.60)	0.17 (1.19)*	-0.04 (-0.13)	0.27 (0.78)*	-0.03 (-0.18)	0.17 (1.22)*	0.04 (0.52)	0.09 (1.58)*
X2	0.22 (0.90)*	0.69 (2.77)**	0.48 (0.98)*	1.38 (2.66)**	0.24 (0.93)*	0.61 (2.93)**	0.11 (0.82)*	0.31 (3.08)***
X3	-0.19 (-0.80)*	0.03 (0.13)	-0.22 (-0.45)	0.17 (0.33)	-0.14 (-0.55)	-0.06 (-0.28)	-0.11 (-0.86)*	-0.04 (-0.377)
X4	-0.25 (-1.10)*	0.24 (1.01)*	-0.42 (-0.88)*	0.43 (0.85)*	-0.27 (-1.03)*	-0.14 (0.67)	-0.15 (0.23)	0.08 (0.83)*
X5	0.37 (1.96)*	0.38 (1.90)*	0.80 (2.44)**	0.54 (1.54)*	0.43 (2.39)**	0.22 (1.56)*	0.19 (1.87)*	0.15 (1.93)*
X6	0.03 (0.17)	0.15 (0.96)*	0.06 (0.21)	0.24 (0.85)*	0.60 (0.42)	0.14 (1.24)*	0.03 (0.73)	0.09 (1.41)*
X7	-0.18 (-1.0)*	-0.02 (-0.10)	-0.43 (-1.28)*	0.08 (0.22)	-0.23 (-1.24)*	0.07 (0.46)	-0.09 (-0.97)*	0.00 (0.05)
X8	-0.19 (-0.72)	0.16\ (0.59)	-0.32 (-0.86)*	0.20 (0.50)	-0.13 (-0.65)	0.06 (0.38)	-0.07 (-0.50)	0.06 (0.50)
R^2	0.15	0.34	0.20	0.31	0.20	0.34	0.14	0.37

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Error term	1.47	1.63	1.49	1.65	1.51	1.77	1.49	1.76
F statistics	0.77	2.20**	0.99	1.96*	0.99	2.26**	0.72	2.52**

****, **, * = significant at 1%, 5% and 10% respectively*
R^2 = coefficient of determinant
Values in parenthesis are t-statistics of individuals X variables
X = Independent variable (X1 = level of education, X2 =farm size, X3 = regression for antimicrobial use, X4 = duration of administration, X5 = who makes the prescription, X6 = reasons for treatment using antimicrobials, X7 = frequency of consulting a veterinarian, X8 = availability of veterinarian when needed)
Y = Dependent variable (Y1 = the most common antimicrobial use, Y2 = frequency of use)

IV. Discussions

Information on the occurrence of antimicrobial resistance is needed at the local, national and international levels to guide policy and detect changes that require intervention strategies. Such monitoring programs should be continuous and standardized, enabling comparison between countries as well as overtime. Comparing different antimicrobials, we have shown that resistance gene abundance and penetration on average are higher for drugs used in animals, even when compensating for differences in many resistance genes are known. This is consistent with expectations from previous research into a "farm-to-flock" connection (Marshall and Levy, 2011).

We first analyzed some general trends such as the connection between the use of antimicrobials in animal husbandry and the spread of resistance, previously suggested from studies of one or a few antimicrobials at a time (Bager *et al*., 1997). We observed a clear and significant increase in resistance gene abundance both for antimicrobials approved for animal use and for older antimicrobials that have been longer in the market. These effects are independent and hold even when controlling for differences in number of genes active against each antimicrobial class or subclass. The Danish antimicrobial resistances, on the other hand, has a relative bias toward bacitracin and vancomycin and to a lesser extended toward streptomycin, spectromycin and chloramphenicol. Notably, a vancomycin analog (avoparcin) has been previously administered to animals in Europe (Barton, 2000), and was subsequently banned as its use was linked to a rapid European increase in vancomycin-resistant enterococci (VRE) (Aarestrup, 2012).

In Tables 1 and 2, there was a positive correlation between the use of antimicrobials and the occurrence of antimicrobial resistant bacteria in poultry and pig farms. These correlations were significant at both 0.01 and 0.05 levels. For instance, in Table 1, increase in the frequency of antimicrobial use leads to the development of antimicrobial resistance to *E. coli*. In the poultry farms, increase in the frequency of use and dosage of antimicrobial leads to antimicrobial resistance to *E. coli* and Enterococcus. This is in agreement with comparative study done by de Jong *et al*; (2012) and Borg (2012) showing that resistance potential correlates significantly with out-patient antimicrobial use.

To further investigate the effect of agricultural use of antimicrobial on the antimicrobial resistance (Table 3); we collected data on level of education, farm size, reasons for antimicrobial use, duration of administration who makes the prescription, reason for treatment using antimicrobials, frequency of consultancy a veterinarian,, availability of veterinarian when needed. The linear regression coefficient between Y1 and X1 indicated that a unit increases in the most common antimicrobial use, and this was significant at 5%. Thus level of education is a determinant factor in the use of antimicrobials. In Table4, the linear regression coefficient between Y1 and X1 indicated that a unit increases in level of education leads to 0.08 increases in the most common antimicrobial use, and this was not significant. Similarly, as farm size increased, frequency of antimicrobial use increased in pig farms and this was significant at 1%. Samples from some animal species are, on average, more similar in their antimicrobial resistance potential to samples from different animals species, and this similarly does not decrease noticeably with time. This is consistent with earlier research on individual antimicrobials (Johnson *et al*; 2011) showing that resistance determinants, once introduced into the microbial flora, can persist for a long time at low abundance, which might also explain the high vancomycin resistance potential in the Danish population despite its animal-use analog being banned since 1995 (Aarestrup, 2012).

Thus, we conclude that the use of antimicrobials in animals contribute to resistance development in commensal bacteria. Thus, the outcome of our investigation covering a vast range of antimicrobials should provide a profound molecular basis for the ongoing debate on the appropriate use of antimicrobials in agriculture and medicine.

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Primary Pre-Sacral Carcinoid Tumor: A Rare Entity

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Abstract- Carcinoid tumors are commonly found in the gastrointestinal tract and are rarely seen in the presacral/sacrococcygeal region. Moreover, such tumors at these sites are usually silent without associated carcinoid syndrome even if the tumor has metastasized. These tumors may arise in tailgut cysts or teratomas thereby suggesting their congenital origin.

Keywords: carcinoid, tailgut cysts, teratomas.

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Primary Pre-Sacral Carcinoid Tumor: A Rare Entity

Manisha Sharma [α], Manas Madan [σ], Mridu Manjari [ρ] & Saumil Garg [ω]

Abstract- Carcinoid tumors are commonly found in the gastrointestinal tract and are rarely seen in the presacral/ sacrococcygeal region. Moreover, such tumors at these sites are usually silent without associated carcinoid syndrome even if the tumor has metastasized. These tumors may arise in tailgut cysts or teratomas thereby suggesting their congenital origin.

Keywords: carcinoid, tailgut cysts, teratomas.

I. Introduction

Carcinoid tumors are a group of neuroendocrine tumors (NET), that are so named because of their ability to secrete bioactive hormones. These tumors can be found along the whole length of gastrointestinal tract (GIT) from the foregut, midgut to the hindgut [1].

Pre sacral/sacrococcygeal space is a potential space, that contains multiple embryological remnants and is a site for development of various types of tumors. Chordomas are the commonest among them [2]. Carcinoid tumors are rare at this site and are often silent with no associated carcinoid syndrome even if the tumor has metastatized [1,2].

These tumors may arise within tailgut cysts and teratomas suggesting the congenital nature of these tumors and the fact that they may have their origin from the residual neuroendocrine cells within hindgut remnants [2,3].

Herein, we report a case of 30 year female who presented with vague abdominal and back pain for one year with intermittent constipation. Biopsy was performed which showed features of carcinoid tumor.

II. Case History

A 30 year-old female was admitted to the hospital, who presented with complaints of vague abdominal and back pain for one year along with constipation on and off. Magnetic resonance imaging (MRI) showed a well defined lobulated soft tissue mass 8.4x7.0x5.4 cm, involving the pelvis with both cystic and solid components. The mass was abutting the sacrum and reaching up to the aortic bifurcation. Rectum and urinary bladder were normal. No lymphadenopathy was observed (Fig 1).

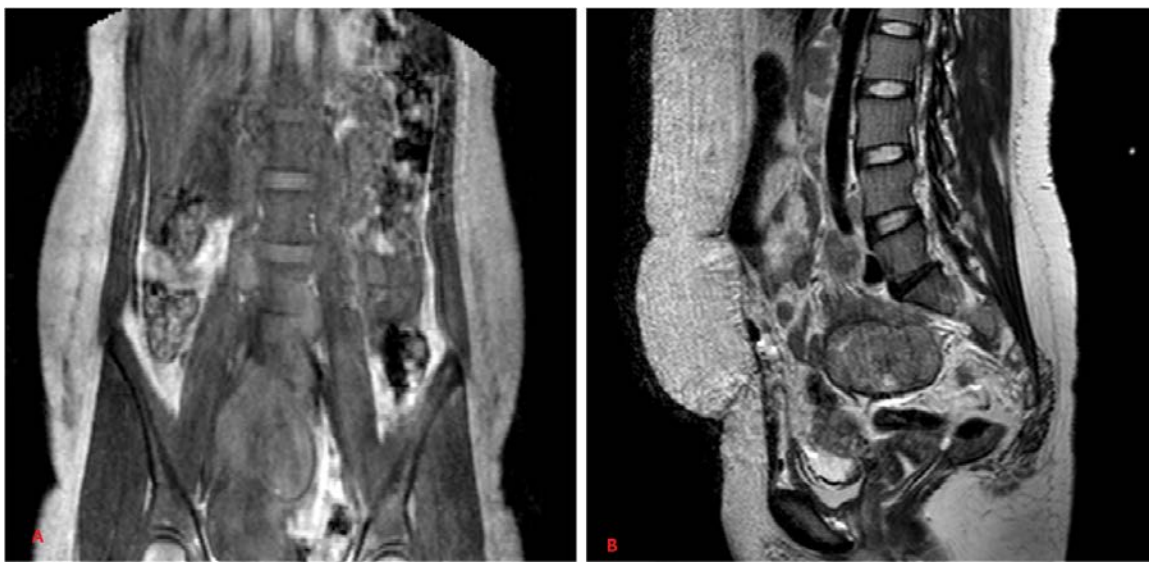

Figure 1 (A,B) : Well defined lobulated soft tissue mass 8.4x7.0x5.4 cm, involving the pal vis with both cystic and solid components, abutting the sacrum and reaching up to the aortic bifurcation

Biopsy of the mass was performed. Histopathology showed fibromyxoid and fibrillary background, in which were present tumor cells arranged were round to oval with moderate to abundant pinkish, granular cytoplasm and indistinct cell outlines. The nuclei were round to oval and eccentric. Vascular

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channels were also seen encircling small groups. Occasional rosette formation was also appreciated. Diagnosis of neuroendocrinal tumor? Carcinoid was made and immunohistochemistry (IHC) advised. IHC showed immunoreactivity for chromogranin and cytokeratin (CK7). (Fig 2).

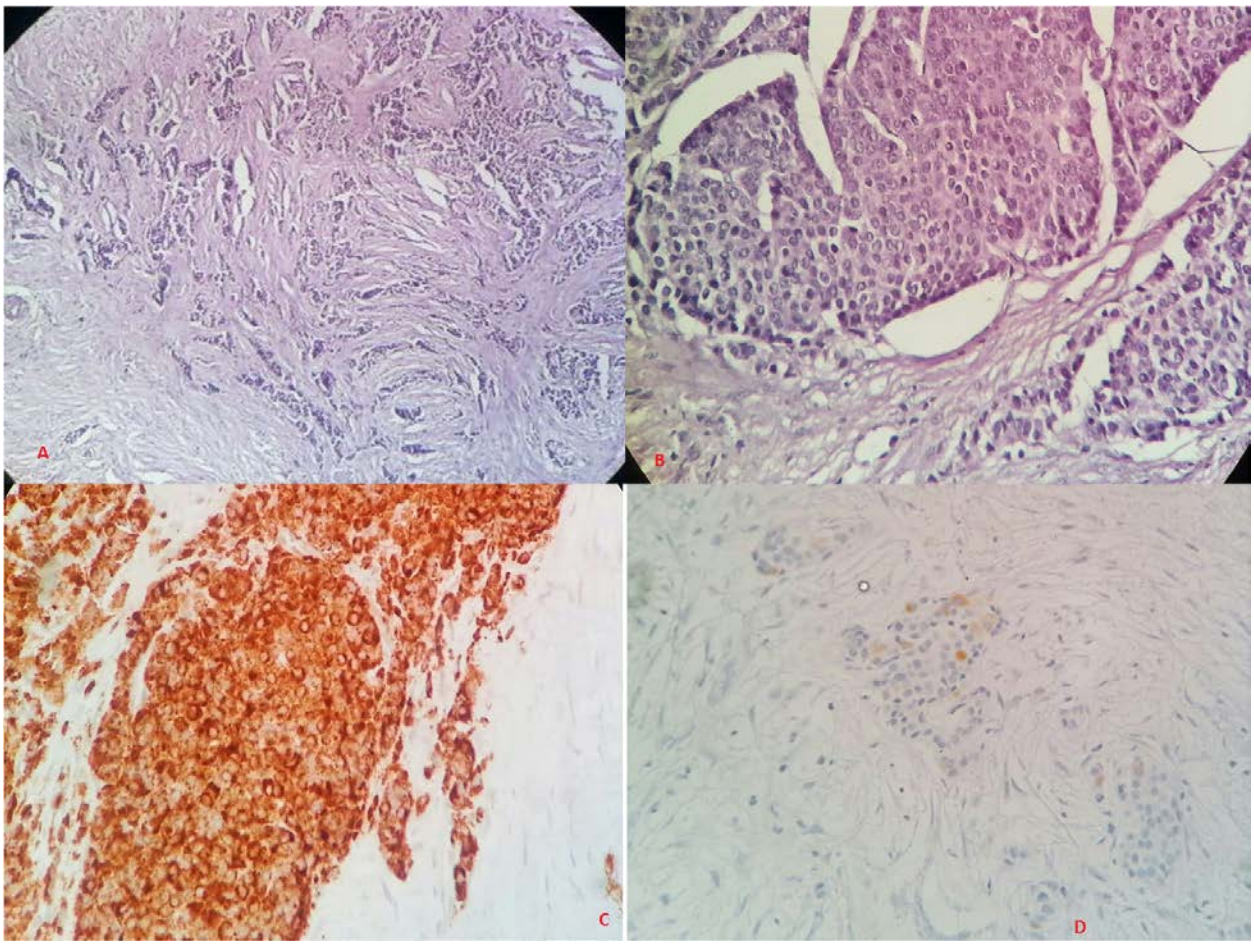

Figure 2 (A) : Scanner view showing the individual tumor cells arranged in insular pattern, nests and cords. (H&E, 40x)

Figure 2 (B) : Low power view showing the individual tumor cell which are round to oval with moderate to abundant pinkish, granular cytoplasm and indistinct cell outlines. (H&E), 100x)

Figure 2 (C,D) : IHC showing positive immunoreactivity for chromogranin and cytokeratin (CK7) respectively. (100x)

So, a final diagnosis of primary presacral carcinoid tumor was made as MRI showed no other mass lesion in the GIT. Transabdominal en bloc resection of the tumor was performed.

III. Discussion

NET s are commonly found in the GIT and are referred to as gastroenteropancreatic (GEP) NETs [1,4]. These are most often seen in the small intestine, rectum and appendix in that order. These tumors are rarely found in the presacral region and when present are usually associated with tail gut cysts (TGC) [1,5,6].

TGCs, also known as retrorectal cystic hamartomas are remnants of embryonic primitive hindgut and present as multiloculated cysts in presacrococcygeal space (1,2,3,7). Persistence of this embryological remnant results in the development of TGC[3]. These cysts can undergo malignant transformation to adenocarcinomas, carcinoid tumors, squamous cell carcinoma and sarcomas [3,7,8,9,10,11,12].

These tumors usually show female preponderance, which suggests possible hormonal influence in the pathogenesis[2,3]. Presacral carcinoid tumors usually are asymptomatic and produce symptoms only related to mass effect i.e. pelvic pain, rectal fullness and constipation. Other potential manifestations include infection, fistula formation, bleeding and malignant transformation. Typical symptoms associated with carcinoid tumors i.e. flushing, sweating, hypertension, watery diarrhea are not seen in these tumors. This behavior simulates the carcinoid tumors arising in colon and rectum that also tend to be silent [1,2,3]. The patient in this reported case was a 30 year female who presented only with vague abdominal pain and constipation.

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CT scan/MRI are useful to identify the primary tumor located at the presacral region and to plan pre-operatively in order to delineate the pelvic structures which are in close proximity to the tumor [1,2,13].

Presacral carcinoids are histologically similar to those arising in any other location regardless of the clinical features that the patient presented with[1,3]. Immunohistochemically, these tumors express cytokeratin (CK) and one or more of the neuroendocrine markers (chromogranin, synaptophysin, neuron specific enolase) [3]. In this case too, histopathology showed typical carcinoid morphology and IHC revealed positivity for CK7 and chromogranin.

Similar to all GIT carcinoids, presacral carcinoids can also metastatize to regional lymph nodes, liver, lungs and bones with risk increasing with the size, nodal status and histological growth pattern [1,14.15].

The prognosis of these tumors depend on tumor histology, size of the tumor, metastasis and performance of complete tumor resection [2,3].

IV. Conclusion

Carcinoid tumors in presacral region are rare and do not differ clinically and histologically from those arising in colon and rectum. However, these are less aggressive and more localized. These tumors should be included in the differential diagnosis of presacral mass and close follow up should be maintained for early diagnosis and management of recurrence or metastasis.

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Risk Factors Associated with Acquisition of ESBLEscherichia Coli Infection, Detection and Treatment, a Case Report

By Dr. Gadangi Indira
Kakatiya University, India

Abstract- ESBL group of organisms are beta lactamase enzyme producing organisms capable of breaking the beta lactam ring in antibiotics hence are resistant to usually cephalosporins and few other antibiotics. In these *E.coli* is the most common bacteria that lives in gut harmlessly but causes Urinary tract infection and in severe cases blood poisoning, septicemia or bacteremia leading to serious sepsis. When not treated it leads to inflammation of body parts, blood clots, blocking oxygen supply and ultimately causing death. In present study report a 51 years old Indian tourist patient was admitted in a Wake Med Health hospital at USA, with symptoms of UTI.In hospital she was diagnosed with ESBL *E.coli*UTI infection with>100,000 colonies /ml and blood culture showed positive result. In this case the Sepsiswas resulted as secondary infection. She even suffered with chronic anemia. The previous medical history of subject showed several risk factors for acquisition of infection. These include elder age, female gender,chronic anemia, recent hospitalization, surgical procedure (due to hysterectomy), intravenous catheterization, intensive careand prolonged usage of high potency antibiotics.All these factors are established as predictive and prognostic risk factors for acquisition of infection and also results in colonization of organism.

Keywords: ESBL, escherichia coli, CLSI, MIC method, PICC line cephalosporinsand ertapenem.

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Risk Factors Associated with Acquisition of ESBL*Escherichia Coli* Infection, Detection and Treatment, a Case Report

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Abstract- ESBL group of organisms are beta lactamase enzyme producing organisms capable of breaking the beta lactam ring in antibiotics hence are resistant to usually cephalosporins and few other antibiotics. In these *E.coli* is the most common bacteria that lives in gut harmlessly but causes Urinary tract infection and in severe cases blood poisoning, septicemia or bacteremia leading to serious sepsis. When not treated it leads to inflammation of body parts, blood clots, blocking oxygen supply and ultimately causing death. In present study report a 51 years old Indian tourist patient was admitted in a Wake Med Health hospital at USA, with symptoms of UTI.In hospital she was diagnosed with ESBL *E.coli*UTI infection with>100,000 colonies /ml and blood culture showed positive result. In this case the Sepsiswas resulted as secondary infection. She even suffered with chronic anemia. The previous medical history of subject showed several risk factors for acquisition of infection. These include elder age, female gender,chronic anemia, recent hospitalization, surgical procedure (due to hysterectomy), intravenous catheterization, intensive careand prolonged usage of high potency antibiotics.All these factors are established as predictive and prognostic risk factors for acquisition of infection and also results in colonization of organism. The antibiotic sensitivity test was done by using CLSI, MIC method on Ampicillin, Cefazolin, Cefepime, Celfazidine, Celtriaxone, Ciprofloxacin, Levofloxacin, Tobramycin showed resistant, Nitroflurantoin showed semi resistant and Ertapenem, gentamicin,Amikacin showed susceptibility. Hence the subject was treated with Doripenemas Intra Venous administration for 15 days with the help of a peripherally inserted central catheteri.e., PICC line.In this case study report,the excessive usage of high dose antibiotics for longer period made the organism resistant or immune. This factor was considered as the primary risk factor followed by hospitalization and gender. In conclusion the study of risk factors help in identification of high-risk cases of UTI positive infection. But still individualization is needed for identification of risk factors.The drug used for the treatment is expensive and often not available in developing countries. The drug sensitivity tests helps in establishing an empirical antibiotic policy.

Keywords: ESBL, escherichia coli, CLSI, MIC method, PICC line cephalosporinsand ertapenem.

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I. Introduction

ESBL group of organisms are beta lactamase enzyme producing organisms capable of breaking the beta lactam ring in antibiotics hence are resistant to usually cephalosporins and few other antibiotics. The emergence these ESBL microorganisms are seen more from the last two decades only. In these *E.Coli* is the most significantbacteria that lives in gut harmlessly but causes community acquired Urinary tract infection (2) and in severe cases blood poisoning, septicemia or bacteremia are resulted (6,13) leading to serious sepsis. The rate of mortality is also recorded high in ESBL *E.coli* septicemia than other infections (1) and if not treated it leads to inflammation of body parts, blood clots, blocking oxygen supply ultimately causing death. The literature available on the epidemiology of these infections is inadequate as most of studies are mainly focused on UTIs and bacteremia. Due to the worldwide increasing incidence of ESBL *E.coli* infection, the study of clinical risk factors is necessary to develop infection management approaches for prevention. Furthermore the therapeutic options are very limited for these infections as these bacteria are resistant to most of the antimicrobial drugs. Hence this paper mainly focused on a case report of anadult female patient who acquired the *E.coli* Bacteremia and admitted in hospital for treatment. The study of this case is appropriate enough to establish an empirical antibiotic or antimicrobial policy.

a) Case report

A 51 years old female patient was admitted in Wake Med Hospital, in North Carolina, USA with symptoms of high fever, chills, headache, recurrent vomiting and body rash. She is an Indian Microbiologist and was visiting America on vacation. She went to Emergency Department for fever and vomiting. Her body temperature was 104°F, but pulse rate and Blood Pressure was recorded normal. Cultures were obtained and patient was note to have pyuria. The subject was discharged on Levaquin. The patient did not get better and continued to feel feverish and had vomiting. As the blood cultures come out positively, she was asked to come to the emergency department for re evaluation. Urine analysis again showed findings consistent with

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Urinary Tract Infection. The subject was then treated with IV Rocephin, and was admitted for further evaluation and management.

The interim diagnosis stated that she has ESBL E.coli sepsis, ESBL E.coli UTI, chronic anemia, Iron deficiency, Vitamin B12 deficiency and rash on back and right forearm. The ancillary data in laboratory showed Sodium-137, Potassium-3.6, Chloride-108, Bicarbonates-24, BUN-7, Creatine-0.69, Glucose-107, Calcium-8, AST-24 from 75, ALT-42 from 67, Alkaline phosphatase-140, Albumin-3, TSH-1.71, Ferritin-49, Iron-15, TIBC-275, Vitamin B12 of 94, Folate 11.3, WBC Count-5.5, HB-7.9,Platelet count-239,000. Hepatitis panel was negative.

b) Cultures

Blood cultures from 2nd and 3rd day showed negative result but first day of admission showed positive ESBL *E.coli* sepsis. Urine cultures from the day one showed positive result.

c) Diagnostics

The chest X-ray on second day of admission, negative study for infection and KUB showed no acute abnormalities. Ultra sound bilaterals showed normal kidneys with some debris in the bladder. Hence all the vitals organs were stable and functioning properly. As the clinical laboratory examinations of blood and urine samples showed acute UTI of ESBL *E.coli* with >100,000 colonies/ml of urine and blood cultures positive, she was referred to Infectious disease doctor for management of the infection. The gram-negative sepsis caused by ESBL *E.coli*, likely source secondary to urinary tract infection. Initially the patient was treated with Rocephin. As the blood culture grew ESBL *E.coli*, depending upon the sensitivities, she was treated with Doripenem. Doctor from ID department has guidedin the treatment. The patient, thus far, responded well to the treatment and has been afebrile, with normal white blood cell count. Vomiting and fever has subsided.

For acute anemia work up showed vitamin B12 deficiency hence she was treated with Iron sulphate as well as vitamin B12-1000mcgs IM. Shehas received with three shots of vitamin B12. The skin rash present at the time admission has much improved and it was of unclear etiology.

II. Methodology

The blood and urine samples were collected aseptically and subjected for culturing. Identification of microorganism was done on the basis of morphological features and biochemical tests. After detection the antimicrobial and susceptibility assay was performed on Ampicillin, Cefazolin, Cefepime, Celfazidine, Celtriaxone, Ciprofloxacin, Levofloxacin, Tobramycin, Nitroflurantoin, gentamicin, Amikacin and Ertapenem by CLSI, M7-A microdilution MIC method.

III. Results

By critical analysis of patient previous history, so many risk factors were noticed for acquisition of infection. The factors associated were i) Elder Age ii) Female gender iii) working atmosphere iv)recently underwent surgery v) admission in Intensive care unit due to surgical procedure and longer hospitalization prior to infection vi) intravenous catheterizationvi) prolonged usage of high potency antibiotics and vii) acute anemia.

The antimicrobial and susceptibility assay was performed on Ampicillin, Cefazolin, Cefepime, Celfazidine, Celtriaxone, Ciprofloxacin, Levofloxacin, Tobramycin, Nitroflurantoin, gentamicin, Amikacin, Ertapenemand Imipenem. As shown in Table-1, the bacteria showed total susceptibility to Amikacin, Ertapenem, Gentamycinand Imipenem whereas these showed intermediate susceptibility to Nitrofurantoin. The bacteria exhibited totalresistance to Ampicillin, Cefazolin, Cefepime, Celfazidine, Celtriaxone, Ciprofloxacin, Levofloxacin, Tobramycin.

Table 1 : Antibiotic sensitivity (MIC) test (courtesy by hospital authorities)

S.No	Antibiotic	Susceptibility test
1	Ampicillin,	Resistant
2	Amikacin	Susceptible*
3	Cefazolin	Resistant
4	Cefepime	Resistant
5	Celfazidine	Resistant
6	Celtriaxone	Resistant
7	Ciprofloxacin	Resistant
8	Levofloxacin	Resistant
9	Tobramycin	Resistant
10	Nitroflurantonin	Intermediate
11	Gentamycin	Susceptible*
12	Ertapenem	Susceptible*
13	Imipenem	Susceptible*

[Indicating the susceptible antibiotics]*

IV. Discussions

The prevalence of ESBL infections is increasing rapidly from the last two decade only (10). There is a limited detailed epidemiological data was recorded as the cases are reported as out patients in hospital, in many countries. (3,7). Only a few authors have studied the risk factors associated in acquisition of ESBL infection. But to formulate the effective strategies to prevent the outbreak of these ESBL infections as community acquired infections, the study of risk factors involved in acquisition infection is essential.

However there are several significant studies in identifying the risk factors, the data recorded for each patient is independent and has lot of disparity. This

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disparity may be attributed to the difference in epidemiological outbreaks as well as lack of correlating the risk factors in identifying the colonization of these bacteria.

In the present case report the risk factors listed as female gender, elder age, work atmosphere, previous history of hospitalization, past history of IV catheterization, preceding history of uterine surgery, exposed to high dose of antibiotics usage and travel are the predictive risk factors for acquiring the ESBL *E.coli* infection (11,14). Ena et al 2006 (5)in their epidemiological study report has attribute elder age as a risk factor for acquisition of *E.coli* infection. Even the colonization of these bacteria in adults is high rather than younger ones. (15). As the subject is a microbiologist there is more chance of colonization. The females are more prone to UTI as the males have longer course of urethra and even prostratesecretions show bacteriostatic properties.

The IV and UT catheterization has significantly associated in promoting the ESBL infection (4). Even the surgical procedures involving the urinogenitalorgans are also an independent risk factor in this case reports. The studies by Rodriguez-Bano J 2004 (14) and Ena J 2006 (5) have corroborated with this risk factor. According to the study report of PairojSaonuam et al 2008 (12), prior usage of antibiotics that too third generation cephalosporins is an important risk factor associated with ESBL infection.

The administration of effective drug is selected basing on the antibiotic sensitivity test and drug of choice in this case report is the doripenem or ertapenem. Several study reports have recognized penem drugs as the choice of treatment for treating the infections caused by ESBL producing isolates (8)). These are most commonly administered drugs to treat the outbreaks of infection. The subject was responded and became healthy by administrating longer duration of IV antibiotic course by PICC line (peripherally inserted central catheter) therapy, after discharging from hospital.

V. Conclusion

The evaluation of risk factors in acquisition of ESBL *E.coli* infection help in identification of high-risk cases of UTI positive infection. But still individualization is needed for identification of risk factors. It is essential to study the risk factors for formulating new strategies in prevention of more deadly infectionsas septicemia, caused by ESBL *E.coli*. By studying the sensitivity tests and knowing the drug of choice for the treatment the empirical antibiotic therapy should be established.

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Patterns of Thyroid Lesions: A Histomorphological Study

By VL Ramesh, S Sunitha, R Rupnarayan & C Narayana Murthy
Basaveshwara Medical College, India

Abstract- Background: Thyroid lesions are fairly common in and around Kolar town. This study was undertaken to study the various histomorphological types of neoplastic and non-neoplastic lesions of the thyroid and to correlate these with respect to age and sex.

Methods: All thyroid specimens received at the pathology Department of Sri Devaraj Urs Medical College, Kolar during the period January 2000 to December 2004 were processed. A detailed histomorphological study was done. The histomosphological type was correlated with the age, sex and clinical presentation.

Results: Total 120 cases of thyroid were studied. Most common age group affected was between 3rd and 5th decade. Females were predominantly affected. The non-neoplastic lesions reported in this study were thyroglossal duct cyst 1 case (0.83%), De Quervain thyroiditis 1 case (0.83%), Hashimoto thyroditis 11 cases (9.16%), colloid goiter 7 cases (5.83%), multinodular goiter 35 cases (29.16%), diffuse toxic goiter 2 cases (1.66%). Among neoplastic lesions follicular adenoma 43 cases (35.83%), atypical follicular adenoma one case (0.83%), papillary carcinoma classic variant 11 cases (9.16%), follicular variant of papillary carcinoma 7 cases (5.83%) and one case (0.83%) of medullary carcinoma.

Keywords: goiter, thyroid lesions.

GJMR-C Classification : NLMC Code: WK 200

PATTERNSOFTHYROIDLESIONSAHISTOMORPHOLOGICALSTUDY

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Patterns of Thyroid Lesions: A Histomorphological Study

VL Ramesh [α], S Sunitha [σ], R Rupnarayan [ρ] & C Narayana Murthy [ω]

Abstract- Background: Thyroid lesions are fairly common in and around Kolar town. This study was undertaken to study the various histomorphological types of neoplastic and non-neoplastic lesions of the thyroid and to correlate these with respect to age and sex.

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Conclusion: Total 120 thyroid lesions were studied in the present study out of this 57 cases were non-neoplastic and neoplastic were 63 cases. Sub–acute thyroiditis reported was 0.83%. Studies conducted by others range from 0.15% to 4.25%. Hashimoto thyroiditis reported was 9.16%. Other studies range from 4.25% to 5.68%. Colloid goiter reported was 5.83%. Reports of others range from 49.18% to 36%. Multinodular goiter reported was 29.16%. Other studies reported range from 3.19% to 18%. Diffuse toxic goiter reported was 1.66%. Other study reported incidence of 2.12%. Total neoplastic lesions reported was 52.5%. Benign lesions reported were 36.66%. Of these follicular adenoma constituted 35.83%. A typical adenoma reported was 0.83%. Total malignant lesions reported were 15.63%. Total thyroid malignancies reported by other studies range from 14% to 31.91%. Papillary carcinoma classic variant found was 9.16%, follicular variant of papillary carcinoma reported was 5.83%. Papillary carcinoma reported by other studies range from 7.44% to 61.1%. Medullary carcinoma constituted 5.16%. Other study reported as 6.5% of medullary carcinomas. In conclusion, most common symptoms was neck swelling. Majority patients were between 3rd and 6th decade with female preponderence. Follicular adenoma was the most common pathological lesion. Commonest malignancy was the papillary carcinoma.

Keywords: goiter, thyroid lesions.

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I. Introduction

Thyroid gland is unique among the endocrine glands in having a wide spectrum of diseases ranging from functional enlargements immune-logically mediated enlargements to the neoplastic lesions. These enlargements may be diffuse or nodular at times causing obvious physiological changes. In contrast patient having a papillary carcinoma thyroid with lymph node secondaries may remain asymptomatic till a very late stage. Occasionally a patient may present with obvious metastatic disease with an undetectable primary (occult or hidden malignancy of thyroid).

Thyroid gland lesions appear to be common in and around the city of Kolar. So the classification of various histomorphological types of tumor is important to categorize the lesion into non-neoplastic and neoplastic lesion of thyroid. The WHO published its second edition on the histological classification of thyroid tumors in 1988[1]. Based on WHO we can classify our neoplastic lesions. It will be of great value for clinicians for further therapy and prognosis.

The present study is intended to study the various histomorphological changes of non – neoplastic and neoplastic lesions of the thyroid, as there are no studies on the patterns of thyroid lesions in and around Kolar, which has high number of patients with thyroid enlargements.

II. Materials and Methods

The material for the present study comprised of specimens received at Department of Pathology, Sri Devraj Urs Medical College, Tamaka, Kolar, between January 2000 and December 2004 from patients admitted to R.L. Jalappa Hospital and S.N.R. Hospital, Kolar. All cases registered in our department files for thyroidectomy and diagnosed between January 2000 and December 2004 for a period of five years were reviewed. The period of retrospective study was from Jan 2000 to Dec 2003 and prospective study from Jan 2003 to Dec 2004.

The specimen were fixed in 10% formalin for 24-48 hour. Large specimens were cut serially (at 1cm thickness) before fixing. After fixation, representative areas were selected for paraffin embedding. In case of encapsulated lesions, adequate representation from tumour capsule – thyroid interface was given. Section were cut at 4-5 microns thick and stained with

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heamatoxylin and eosin and studied. This was done for all cases received between January 2003 and December 2004.

Special stains like methyl violet, vanGieson, masson trichrome and congo red were performed for necessary cases.

Stained histopathology slides were studied in detail. All details of the case consisting of clinical history, external examination, gross features, microscopic features and final diagnosis were filled in a proforma. Details from all proforma were tabulated in a master chart.

III. Results

The present study is undertaken for a period of five years between January 2000 and December 2004. Retrospective study for three years from January 2000 to December 2002 (48 cases). Prospective study for two years from January 2003 to December 2004 (75cases).

A total number of 8,638 specimens were received during this period. Of these 123 cases were clinically thyroid neck swellings. Among these 3 cases were excluded, which were histopathologically diagnosed as granulation tissue (Sl.No.28 and 42) and normal lymph node (Sl.No.118). Remaining 120 cases were thyroid lesions and included in this study.

Table 1 : Age and Sex Distribution

Sl.No	Age	No.of. Cases	Male	Female
1	<10	1	-	1
2	10-19	4	-	4
3	20-29	33	4	29
4	30-39	40	2	38
5	40-49	21	5	16
6	50-59	11	1	10
7	60-69	9	-	9
8	70-79	1	-	1
	Total	120	12(10%)	108(90%)

Table 2 : Symptoms with which the patient presented

Sl.No	Sympotoms	No.of.Cases
1	Neck Swelling	120 (100%)
2	Dysphagia	24 (20%)
3	Dyspnoea	15 (12.5%)

Table 3 : External examination of neck swelling had following features

Sl.No	Signs	No.of.Cases
1	Diffuse. a. Sub acute thyroiditis b. Hashimoto thyroidtis c. Colloid goiter d. Diffuse toxic goiter e. Papillary carcinoma	34 (28.3%)
2	Solitary nodule. a.Follicular adenoma b. Atypical adenoma c. Thyroglossal duct cyst d. Papillary carcinoma e. Medullary carcinoma	51 (42.5%)
3	Multiple nodules. a. Multinodular goiter	35 (29.2%)

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Table 4 : Morphologic types of thyroid lesions

Sl.No	Morphologic type	No.of.Cases	%
1	Non - Neoplastic lesions	57	47.5
2	Neoplastic lesions	63	52.5

Table 5 : Histomorphologic types and their incidence in different sex and age groups

SL.No	Age in years	<10		10-19		20-29		30-39		40-49		50-59		60-69		70-79		TOTAL
	Types	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
1	Thyroglossal duct cyst	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
2	Sub- acute thyroiditis	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
3	Hashimoto thyroiditis	-	-	-	-	-	1	-	3	1	2	-	3	-	1	-	-	11
4	Colloid goiter	-	-	-	-	1	2	-	2	-	1	-	-	-	1	-	-	7
5	Multinodular goiter	-	-	-	2	2	6	-	11	1	8	-	3	-	2	-	-	35
6	Diffuse toxic goiter	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	2
7	Follicular adenoma	-	-	-	2	3	12	-	11	2	6	-	3	-	3	-	1	43
8	Atypical adenoma	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
9	Papillary Ca.Classic	-	-	-	-	-	2	1	3	1	-	1	2	-	1	-	-	11
10	Papillary.Ca.Follicular	-	-	-	1	-	1	1	4	-	-	-	-	-	-	-	-	7
11	Medullary Carcinoma	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
	TOTAL	-	1	-	5	6	26	2	36	5	17	1	11	-	9	-	1	120

Table 6 : Histomorphologic types of non-neoplastic lesions of thyroid

Sl.No	Types	No.of.Cases	%
1	Thyroglossal duct cyst	1	0.83
2	Sub- acute thyroiditis	1	0.83
3	Hashimoto thyroiditis	11	9.16
4	Colloid goiter	7	5.83
5	Multinodular goiter	35	29.16
6	Diffuse toxic goiter	2	1.66
	Total	57	47.5

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Table 7 : Histomorphologic types of neoplastic lesions

Sl. No	Types	No of cases	%
1	Follicular adenoma	43	36
2	Atypical adenoma	1	1
3	Papillary.Ca.Classic	11	9
4	Papillary .Ca.Follicular	7	6
5	Medullary Carcinoma.	1	1
	Total	63	(52.5%)

IV. Discussion

Total 120 thyroid lesions were studied in the present study. Of this 57 cases were non– neoplastic and 63 cases were neoplastic consisting of 47.5% and 52.5% respectively. A study conducted by Sankaran[9] reviewed 127 cases and found the percentage of non neoplastic lesions as 85.8% and neoplastic as 14.2% Non-neoplastic lesions, in this study there was one case of thyoglossal cyst (0.83%) out of 120 cases. This was a 5 years old female child. One case of sub-acute thyroid it is was reported (0.83%) in a 38 years female patient out of 120 cases. A study conducted by Arora and Gupta [6,10] reviewed 94 cases and found the percentage of sub-acute thyroditis was 4.25% (4 cases). Another study conducted by Meachim and Young[8] reviewed 1285 cases and found the percentage of sub acute thyroditis was 0.15% (2 cases). Hashimoto thyroditis accounted for 11 cases (9.16%) out of 120 cases. A study conducted by Arora and Gupta [2,6,10] found Hashimoto thyroditis were 4.25% (4 cases) out of 94 cases studied. Another study conducted by Meachim and Young[8] reviewed 1285 cases and found the percentage of Hashimoto thyroditis was 5.68% (73 cases). Total all types of the thyroditis reported were 12 cases (10%) out of 120 cases. Total all types of thyroditis reported in the study conducted by Arora and Gupta[6,10] was 9.57% (9 cases) out of 94 cases. In another study conducted by Meachim and Young[6,8] total all types of thyroditis was 5.99% (77 case) out of 1285 cases studied. Colliod goiter formed 5.83% (7 cases). Maximum cases were in the 3rd to 5th decade of life and one male case was reported. There was a wide range in the incidence of the colloid goiter reported by several authors. In a study conducted by Sankaran[6,9] the incidence of colloid goiter was 36%. The average age being 33 years with female preponderance. In another study conducted by Arora and Gupta[10] the incidence of colloid goiter was 15.95%. In the study conducted by Meachim and Young[8] the incidence of colloid goiter was 49.18%. Mulitnodular goiter was the most common non-neoplastic lesion in this study. There were 35 cases (29.16%) with peak age incidence seen between 3rd and 5th decade of life and was more common in females. In a study conducted by Sankaran[6,9] the incidence of multinodular goiter was 18% and average age incidence was 35 years. In the study conducted by Arora and Gupta[10] the incidence of multinodular goiter was 3.19%. Diffuse toxic goiter accounted to 1.66% (2 cases). Both were female patients. The study by Arora and Gupta[5,10] reported an incidence of 2.12%. Compared to the overall incidence of goiter (all types) in this study (36.65%). Kalpatrick et al[6,11] reported the overall incidence as 39.4 %, predominantly in the 20-49 years age group.

Neoplastic lesions, benign and malignant tumors together formed 63 cases (52.5%) out of total 120 cases studied.

Benign lesions found were in 36.66% (44 cases). Of this follicular adenoma was reported in 35.83%(43 cases). Follicular adenoma was the most common lesion in this study and it was the most common neoplastic lesion. Maximum incidence was seen between 3rd and 5th decade of life with female preponderence. Five male patients were reported. In a study conducted by Arora and Gupta[1,3,7,10] represents 36.17% of follicular adenoma out of 94 cases studies. In another study conducted by Thomas[12] follicular adenoma represented 21.3% out of 121 cases studied.

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The comparison between different histological sub-types of follicular adenoma in this study with incidence reported by various authors is shown below:

Types		Present study	Arora and Gupta[7,10]	Thomas[6,12]
		(43 cases)	(94 cases)	(34 cases)
Micro follicular (foetal)	-	03	05	02
Macrofollicular (colloid)	-	25	27	56
Normofollicular (simple)	-	15	Nil	29
Hurthle cell adenoma	-	Nil	02	02.

Atypical adenoma was found in one case (0.83%). This was female patient aged 27 years.

Malignant tumors (19 cases) constituted 15.63% of the total 120 cases studied.

In contrast, Sankaran[9] reported an incidence of 14%. Arora and Gutpa[7,10] reported an incidence of 31.91% and Thomas[3,5,12] reported an incidence of 19%. Papillary carcinoma classic variant constituted 9.16%(11 cases). Most cases were aged 40 years and below. Two youngest patients were 22 years old females. The oldest patient was a 65 years female with lymph node metastasis. There were only three male patients.

Comparative analysis of histological types of the thyroid carcinoma

Sl. No	Types	Arora & Gupta [10] (94cases)	Thomas [12] (23cases)	Woolner et al [13] (885 cases)	Burn & Taylor [14] (152 cases)	Present Study (19 case)
1	Papillary .Ca	23.33%	34.8%	61.1%	28.5%	94.73%
2	Follicular .Ca	63.33%	60.8%	17.7%	28.5%	-
3	Follicular + Papillary .Ca	-	4.4%	-	-	-
4	Medullary .Ca	-	-	6.5%	-	5.26
5	Anaplastic .Ca	13.33%	-	14.7%	43	-

V. Conclusion

Most of the patients presented with a symptoms of neck swelling. Majority of the patients were between 3rd and 6th decade.Females were predominantly affected.

The commonest lesion was follicular adenoma followed by multinodular goiter.

Most common malignant lesion was papillary carcinoma.

The present study was undertaken to review the recent literature in recognising the histomorphologic criteria for the thyroid lesions and to correlete the histomorphological type of thyroid lesion with age and sex of patient in and arround Kolar town. The drawback of this study was that the present data being hospital generated cannot be regarded as representative of the incidence of thyroid lesion in the general population.

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The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

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(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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References

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- To the point depiction of the research
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- Explain materials individually only if the study is so complex that it saves liberty this way.
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What to keep away from

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- Leave out information that is immaterial to a third party.

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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.

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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