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Papillary Thyroid Carcinoma

Prognosis of Papillary Thyroid

Highlights

Factors of Hepatitis B Virus

Distribution of Genital Mycoplasmas

Discovering Thoughts, Inventing Future

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Role of CK-19, HBME-1 and Galectin-3 in Prediction of Prognosis of Papillary Thyroid Carcinoma – An Experience from a Tertiary Care Centre

By Dr. Samir Ranjan Bhowmik, Dr. Keya Basu, Dr. Subhrojyoti Karmakar, Dr. Ananya Biswas, Dr. Moumita Sengupta, Dr. Sriranjan Mukherjee & Dr. Sujoy Ghosh

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Keywords: *papillary thyroid carcinoma (PTC), total tumor diameter (TTD), cytokeratin 19, prognostic markers.*

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Role of CK-19, HBME-1 and Galectin-3 in Prediction of Prognosis of Papillary Thyroid Carcinoma – An Experience from a Tertiary Care Centre

Dr. Samir Ranjan Bhowmik [α], Dr. Keya Basu [σ], Dr. Subhrojyoti Karmakar [ρ], Dr. Ananya Biswas [ω], Dr. Moumita Sengupta [¥], Dr. Sriranjan Mukherjee [§] & Dr. Sujoy Ghosh [χ]

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Results: Positive expression of CK19 and GAL-3 were correlated significantly with the total tumor diameter and capsular invasion. Positive expression of HBME-1 was correlated significantly with capsular invasion and lymph nodal metastasis. But none of them was found to be associated with age and sex of the patient.

Conclusion: High expression of CK19, GAL-3 and HBME-1 are found to be associated with high volume of total tumor diameter as well as capsular invasion and lymph nodal metastasis. Thus, these IHC markers could be used to assess the aggressive behavior of PTCs.

Keywords: papillary thyroid carcinoma (PTC), total tumor diameter (TTD), cytokeratin 19, prognostic markers.

I. Introduction

Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm of thyroid follicular epithelium.[1] Its incidence has increased dramatically in past few decades reaching up to 80% of malignant thyroid tumors.[2,3,4] Despite the propensity for lymphovascular invasion, majority of the patients with this tumor, if properly treated, have an excellent long-term prognosis.[5] But appropriate treatment primarily depends on the ability of the pathologist to make an accurate diagnosis. Historically, diagnosis of PTC was based on the presence of the papillary architectures but currently it also includes the presence of nuclear features i.e. nuclear overlapping, optical clearing, macronucleoli, irregular contours with pseudoinclusion and grooving.[6] Identification of these features remains difficult and controversial when they are present focally, thus distinction of PTC from other thyroid lesions i.e. benign papillary hyperplasia, some forms of thyroiditis, variants of PTC may not be possible. In this regard, implication of immunohistochemical (IHC) markers are very useful. In this study, we have used a panel of three IHC markers – Cytokertain 19 (CK-19), Hector Battifora Mesothelial-1 (HBME-1) and Galectin-3 (GAL-3) to show their role as prognostic markers of PTC.

II. Materials and Methods

We have conducted a single institution based, retrospective study at a tertiary care centre of Eastern India. Patients with histologically confirmed papillary thyroid carcinoma treated between January 2017 and June 2018 were identified from the Department of General Surgery. All patients had undergone total thyroidectomy for the primary tumor. A total of 41 patients were reviewed for their clinical and pathological data. We have considered gender, age, total tumor diameter (TTD), capsular invasion and lymphnodal metastasis as the parameters to be studied. We have correlated the expressions of CK 19, GAL-3 and HBME-1 with those parameters to show their role in prognostication of PTC (weak and strong expression both considered as positive expression).

CK19 is a low molecular weight cytokeratin which presents widely in simple epithelia and basal cell layers of stratified epithelium.

HBME-1 is a monoclonal antibody generated against a membrane antigen of mesothelial cells.[7] It

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was originally found in malignant mesothelioma; several investigators showed that HBME-1 play an important role in diagnosis of papillary thyroid carcinoma.

GAL-3 is a member of oligosaccharide selective binding protein family known as lectins which plays an important role in the cell growth, apoptosis, cell-matrix interactions, neoplastic transformation and metastasis; and now it's been considered to be an effective indicator that can be available to distinguish the malignant thyroid nodules from the benign ones.[8]

The aim of present study was to investigate the relationship between the expression of CK19, HBME-1 and GAL-3 and the aggressive behavior of PTCs by correlating immunohistochemical results with the clinical features.

a) Statistical Analysis

The Kruskal–Wallis test was performed for comparisons between multiple groups. The χ^2 test was analysed for categorical evaluation. Correlations were evaluated using Spearman's rank correlation. *p*-value < 0.05 was considered as significant. Statistical software (GRAPHPAD PRISM 5) was used for analysis.

III. Results

In Table 1 we have shown the summary of the clinicopathological traits. Among 41 cases, the female: male ratio was 19.5:1 and 14.63% cases were more than 45 years old. 80.48% cases had total tumour diameter more than 1cm. capsular invasion and lymphovascular invasion were found to be present in 26.82% and 31.70% cases respectively.

In Table 2-4 we have summarized the results showing correlation of the IHC markers with five prognostic factors. All the cases with PTC were divided into positive and negative expression groups.

According to our results in Table 2, positive expression of CK19 was correlated significantly with the total tumor diameter ($p < 0.001$). This finding indicated that the larger volume of the total tumor diameter is more likely to express CK19. Positive expression of CK19 was also correlated significantly with the capsular invasion ($p < 0.007$) which denotes that CK19 positivity stands for more aggressive behavior of PTC. On the other hand, expression of CK19 had no significant relationship with age, sex and lymph nodal metastasis.

In Table 3, we have shown the correlation of expression of Galectin 3 with the prognostic parameters. Similarly expression of GAL-3 was correlated significantly with total tumor diameter ($p < 0.005$) and capsular invasion ($p < 0.007$) in all the PTC cases, whereas its expression was found to be not related to patient's age, sex and lymph nodal metastasis.

In Table 4, correlation between the expression of HBME-1 and the prognostic parameters is shown. Expression of HBME-1 was found to be significantly correlated with capsular invasion ($p < 0.01$) and lymph nodal metastasis ($p < 0.002$).

Table 1: Summary of clinicopathological traits

Parameter	% (No. of cases)
Age (Yr)	
≤ 45	85.36 (35/41)
> 45	14.63 (6/41)
Gender	
F	95.12 (39/41)
M	4.87 (2/41)
Total Tumor Diameter	
≤ 1 cm	19.51 (8/41)
> 1 cm	80.48 (33/41)
Capsular Invasion	
Present	26.82 (11/41)
Absent	73.17 (30/41)
Lymph Node Metastasis	
Present	31.70 (13/41)
Absent	68.30 (28/41)

Table 2: Prediction of some clinicopathological characteristics of PTC based on immunohistochemical expression of CK19

Prognostic Factors		CK 19 (N=41)		P Value
		Positive	Negative	
Mean Age		33.88	34.78	0.76
Sex	Male	2	0	0.53
	Female	25	14	
Diameter	≤ 2 cm	2	12	*<0.001**
	>2 cm	25	2	

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Capsular Invasion	Present	11	0	*0.0071**
	Absent	16	14	
Lymph Node Metastasis	Present	9	4	1.00
	Absent	18	10	

Table 3: Prediction of some clinicopathological characteristics of PTC based on immunohistochemical expression of Galectin-1

Prognostic Factors		Galactin-3 (N=41)		P Value
		Positive	Negative	
Mean Age		33.18	36.14	0.33
Sex	Male	2	0	0.53
	Female	25	14	
Diameter	≤ 2 cm	4	10	*0.005*
	>2 cm	23	4	
Capsular Invasion	Present	11	0	*0.0071*
	Absent	16	14	
Lymph Node Metastasis	Present	11	2	0.1559
	Absent	16	12	

Table 4: Prediction of some clinicopathological characteristics of PTC based on immunohistochemical expression of HBME-1

Prognostic Factors		HBME-1(N=41)		P Value
		Positive	Negative	
Mean Age		35.54	32.29	0.26
Sex	Male	0	2	0.16
	Female	24	15	
Diameter	≤ 2 cm	6	8	0.18
	>2 cm	18	9	
Capsular Invasion	Present	10	1	*0.01*
	Absent	14	16	
Lymph Node Metastasis	Present	13	0	*0.002*
	Absent	11	17	

IV. Discussion

Primary thyroid cancers comprise the largest group among malignancies of the endocrine system. 120,000 new cases are added each year. Thyroid carcinomas usually present in the 40-60 age group. Environmental, genetic and hormonal factors have been considered in the etiology of thyroid carcinomas. Many benign conditions like thyroidal adenomas, multi-nodular goiter, thyroiditis, thyroidal cysts, thyroidal malformations and focal granulomatous diseases occur clinically as solitary nodules and malignancy is found in 0.1-0.2% of these conditions.[9]

Cytokeratin-19 (CK-19) expression in thyroid nodules is in general intense and diffuse in papillary carcinoma and heterogeneous labeling in follicular carcinoma and in follicular adenoma, with nil or low expression in other benign lesions.[10,11] Galectins, especially galectin-3, are suggested to play a role in the pathogenesis of well differentiated thyroid carcinoma, particularly in papillary carcinoma.[12]Therefore, it is one of the markers most commonly used to assist in distinguishing thyroid lesions. Hector Battifora mesothelial-1 (HBME-1) has been demonstrated to be important as a thyroid marker of follicular origin, with greater affinity to malignant lesions when compared to benign lesions.[13]

In general, the prognosis of PTC is favorable and ten-year survival rate for PTCs is greater than 90%.[14] However, about 20% of the differentiated thyroid cancer will present with metastasis. So accurate biomarkers which can predict the aggressive behavior of thyroid carcinoma is critical for clinical management.[15]

Tijana *et al* reported that the CK19 was a useful marker for the identification of PTCs and they suggested that the high expression of the CK19 is a predictor for the aggressive behavior of PTC and could help to identify a particular subgroup of PTCs which had a potentially worse prognosis.[16] GAL-3 could be a important tool for guiding therapeutic decisions in patients with thyroid nodules.[17]

The significance of the biomarkers, such as CK19, HBME-1, GAL3, have been widely explored and debated for the differential diagnosis of thyroid neoplasms but the value of these biomarkers as prognostic factors for PTCs is not clear.[16] Thus, in our study, we attempted to investigate whether the expression of the CK19,HBME-1 or GAL3 is linked to the aggressive behavior in papillary thyroid carcinoma.

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In a study performed by Prasad *et al.* consisting of 85 carcinoma and 21 adenoma cases, all carcinomas showed different percentage and intensity of staining and 24% of adenomas showed poor intensity of staining with HBME-1. It was concluded that HBME-1 was a very useful marker in malignancies arising from follicular cells and its negativity in benign lesions has a specificity of 94%.[18] In a study performed by Pisani *et al.*, specific cytoplasmic staining with galectin-3 was observed in a suspicious cell population in fine needle aspiration biopsy of a thyroid nodule. Occult papillary carcinoma was found in the operation material of this case andgalectin-3 was concluded as a marker of malignancy.[19]

Thus, in our study we can conclude that this triad of IHC markers – CK19, GAL-3 and HBME-1 could be used in the prognostication of papillary thyroid carcinoma. The positive expressions of these markers have been significantly correlated with total tumor diameter, capsular invasion and lymph nodal metastasis in this study.

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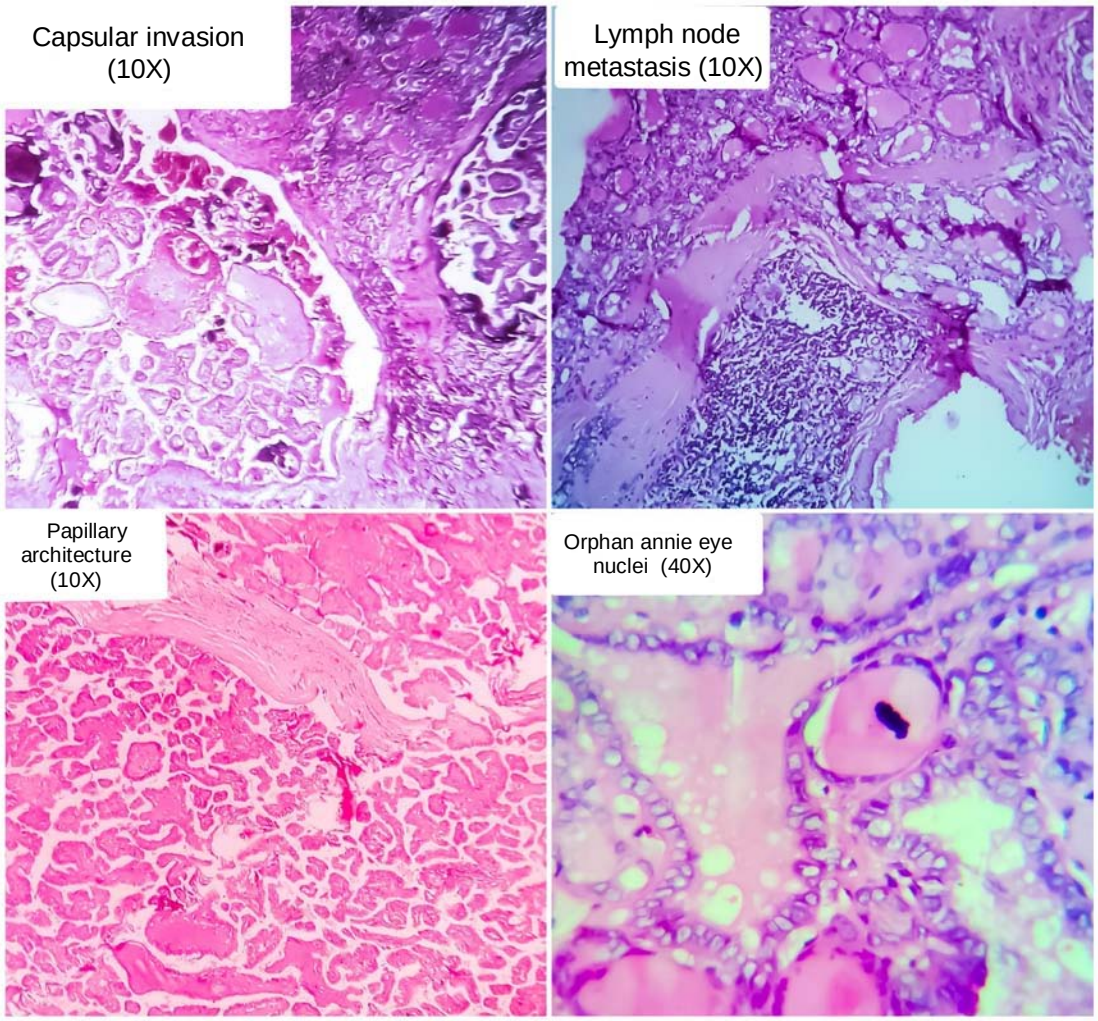


Figure 1

Upper left: Capsular invasion in papillary thyroid carcinoma in H & E (×100)
Upper right: Capsular invasion in papillary thyroid carcinoma in H & E (×100)
Lower left: Papillary architecture in papillary thyroid carcinoma in H & E (×100)
Lower right: Intranuclear clearing in papillary thyroid carcinoma in H & E (×400)

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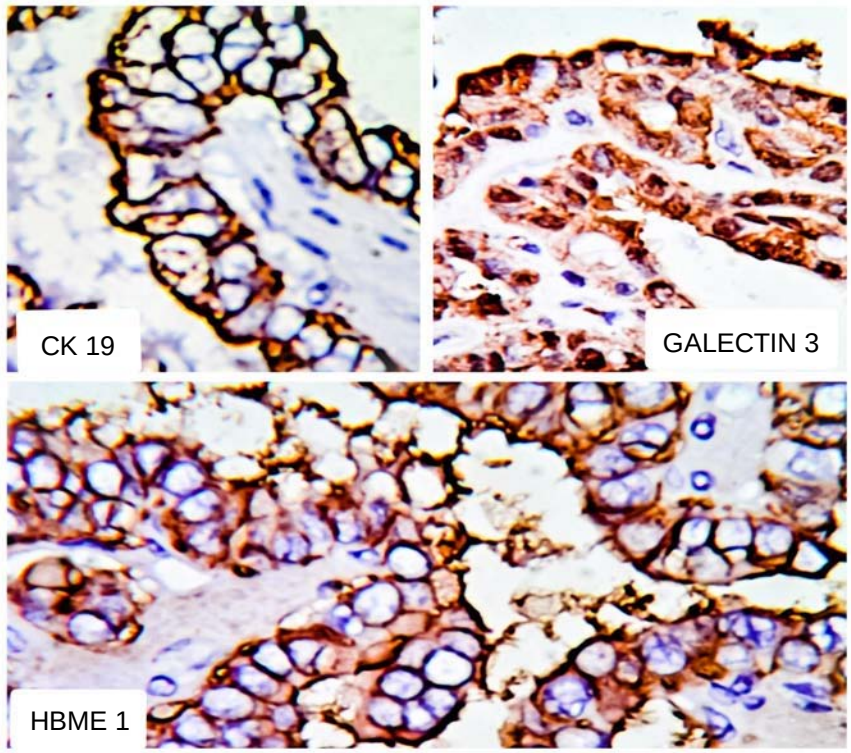


Figure 2

Upper left: CK19 immunostain in papillary thyroid carcinoma (×400)
Upper right: Galectin-3 immunostain
Lower: HBME immunostain in papillary thyroid carcinoma (×400)

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Distribution of Genital Mycoplasmas in pregnant and non-pregnant Women in Iraq of Basrah City

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Abstract- A total of 120 women attending the outpatient clinic of the obstetric and gynecology department of Basrah General Hospital, were evaluated for the role of genital mycoplasmas in certain infectious female genital conditions during the period from December, 1st 2003 to Qctober, 2nd 2004. High vagina and endocervix swabs were cultured. Five species of genital mycoplasmas were isolated and identifiedon modified PPLO medium, the characteristics and biochemical properties of them were fit with those of *Mycoplasma hominis, Ureaplasma urealyticum, M. fermentans, M. genitalium* and *M.penetrans* in which the last three species were identified for the first time in Iraq. *M.hominis, U.urealyticum, M.genitalium* and *M.penetrans* were more frequently distributed in age group 20-39 years, and in women with lower socioeconomic status. A significant difference was found in the isolation of both *M.hominis* and *M. genitalium* from non-pregnant. Also, a significant difference was noted in isolation rates of *M.hominis, M.fermentans* and *M.penetrans* from pregnant women at different gestational periods..

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Distribution of Genital Mycoplasmas in pregnant and non-pregnant Women in Iraq of Basrah City

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Abstract- A total of 120 women attending the outpatient clinic of the obstetric and gynecology department of Basrah General Hospital, were evaluated for the role of genital mycoplasmas in certain infectious female genital conditions during the period from December, 1st 2003 to Qctober, 2nd 2004. High vagina and endocervix swabs were cultured. Five species of genital mycoplasmas were isolated and identifiedon modified PPLO medium, the characteristics and biochemical properties of them were fit with those of *Mycoplasma hominis*, *Ureaplasma urealyticum, M. fermentans, M.genitalium* and *M.penetrans* in which the last three species were identified for the first time in Iraq. *M.hominis*, *U.urealyticum, M. genitalium* and *M.penetrans* were more frequently distributed in age group 20-39 years, and in women with lower socioeconomic status. A significant difference was found in the isolation of both *M.hominis* and *M. genitalium* from non-pregnant. Also, a significant difference was noted in isolation rates of *M.hominis, M.fermentans* and *M.penetrans* from pregnant women at different gestational periods. A statistically significant difference at the level of ($P<0.01$) was found in isolation of both *U.urealyticum* and *M.genitalium* from endocervix region. This study revealed a significant association between the five species of genital mycoplasmas and some cases or symptoms in the female genital tract. Furthermore, different species of bacterial isolates other than mycoplasmas were identified in 12 infected women using contraception. Genital mycoplasmas were found as a single infection in frequent (16.9%) and mixed infection with other causative agents in frequent (17.5%). Also, this study shows the conjunction of *M.hominis* with both *U.urealyticum* and *M.fermentans* in (5.0%), (1.6%) respectively.

I. Introduction

The mycoplasmas are the smallest cell free-life microorganisms, characterized by their small cell size (0.3-0.8μm) and thus can pass through some filters used to remove bacteria. They have the smallest genome size and, as a result, lack many metabolic pathways and lack of a rigid bacterial cell wall (Mayer and Murray etal., 2002). Mycoplasmas are fastidious organisms that require specialized media for their isolation and identification (Razin and Freundt, 1984; Razinetal., 1998). Ultrastructural studies showed that they are constructed of only three organelles: plasma membrane, ribosomes and prokaryotic chromosomes. There is no evidence of any intracellular membrane structure, and because of the lack of rigid cell wall, mycoplasmas are pleomorphic (Lin, 1985). The family Mycoplasmataceae contains two genera that infect humans: Mycoplasma and Ureaplasma, which are usually referred to collectively as mycoplasmas. Seven species of mycoplasmas can be isolated from genitourinary tract, but in female genitourinary tract the most common mycoplasmas isolated are *U. urealyticum* and *M.hominis* have been implicated in human diseases (Cassell and Cole, 1981; Krause and Taylor-Robinson, 1992). *M.hominis* and *U.urealyticum* normally inhabit the urogenital tract in sexually mature men and women. Higher isolation rates are obtained from women than from men, possibly reflecting more favorable growth conditions in the vaginal tract. The degree of colonization with these species is related to sexual activity and to the number of sexual partners (Taylor-Robinson and McCormack, 1979). In 1980 new mycoplasma species were isolated from urethral specimens from 2 of 13 men with nongonococcal urethritis. It was later named *Mycoplasma genitalium* because of the host tissue location, however *M.genitalium* strains were originally isolated from the urogenital tract. This mycoplasma shared several properties with *M.pneumoniae* (Tully et al., 1981,1983; Lind et al., 1984; Taylor-Robinson, 1995).

M.genitalium and *M.penetrans* are recent additions to the pathogenic human mycoplasmas, reviewed by Marmion and Harris, (1996). *M.penetrans*. This newly-identified mycoplasma was isolated by (Lo etal., 1991) from the urogenital tract of Human Immuno Virus of positive homosexual men. In a study in west Africa, *M.genitalium* was associated with nongonococcal urethritis (NGU), particularly in Trichomonas vaginalis negative patients (Pepin et al., 2001).

Preliminary evidence for the involvement of the mycoplasma in the cervicitis and pelvic inflammatory disease was presented by Moller etal. (1984) and Uno etal. (1997). The etiological roles of genital mycoplasmas are: Acute pyelonephritis, bacterial vaginitis, pelvic inflammatory disease, chorioamnionitis, post- abortion and postpartum fever, pneumonia in new borns, non gonococcal urethritis, prostatitis and epididymitis (Mardh and Westrom, 1970; Shepard, 1970, 1980; Taylor-Robinson and McCormack, 1980; Krause and Taylor-Robinson, 1992; Taylor-Robinson, 1996). Furthermore, that mycoplasmas play a role in human infertility (Gnarpe and Fribreg, 1972; Stray-

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Pedersen etal., 1978; Casselletal., 1983; Tothetal., 1983), abortion and stillbirth (Embreeetal.,1980; Kundsin etal.,1981), premature birth and low birth weight of infant (Braun etal., 1971; Embreeetal., 1980; Kundsinetal.,1981).

Despite numerous studies of these organisms, their role in most genital infection remains controversial and illdefined. The ubiquity and low virulence of genital mycoplasmas make it difficult to evaluate their role in producing genital infections, a situation that is further complicated by their frequent isolation together with other sexually transmitted diseases (Watts and Eschenbach, 1988). Furthermore the presence of both genital mycoplasmas (*U.urealyticum* and *M.hominis*) in a large proportion of healthy women complicates the assessment of the pathogenic role of these organisms (Taylor-Robinson and Furr, 1998). However, circum-stantial evidence including high frequency of isolation of mycoplasmas from females with urogenital conditions or infertility warns against minimizing the importance of these microorganisms in human disease (Kundsin and Driscoll, 1970).

Mycoplasmas were the causative agents of disease in human (Krause and Taylor-Robinson, 1992) over many years passed. Despite, numerous studies conducted in Iraq on different genera of bacteria, there have been few studies on the role of genital mycoplasmas in the infectious disease (Simhairi, 1990; Al-Bahli, 1993).

II. Materials and Methods

a) Study population

The population under study was women attending the outpatient clinic of obstetric and gynecology department of Basrah General Hospital. The samples obtained consisted of pregnant women at different gestational periods and non pregnant women including women using contraceptive devices besides women not using contraceptive devices and those with infertility and those with various complaints. Also women presented with different types of abortions are included. The samples were selected randomly from the women above and collected from different parts of Basrah. A total number of two hundred and twenty two women (222) were investigated. Their age ranged from 17 to 50 years. The investigation period extended from 1st of December, 2003 to 2nd of October, 2004. A control group consisted of one hundred (100) healthy women.

b) Collection and Inoculation of samples

Two swabs one from endocervix and the other from high vagina were obtained from each woman and each was inoculated. Onto a suitable medium. A sterile speculum was used. All swabs were transported to the laboratory within (one) hour for culture. For the isolation of mycoplasmas, each specimen was directly inoculated into the liquid phase, mixed up well and tilted for a while, once or twice, to cover the upper slanted portion in a simple monophasic - diphasic culture setup (MDCS) prior to incubation (Al-Sulamietal.,2002). For the isolation of bacteria other than mycoplasmas another two swabs from endocervix and high vagina were obtained from the same women included in the study. Then, each specimen after being transported to the laboratory was directly cultured onto MacConkey& Blood agar by the streaking method then incubation follows.

c) Culture Media

Media for isolation and identification species of genital mycoplasmas were applied depending on Marmion and Harris [5].

d) Cultivation and isolation of mycoplasmas (genital mycoplasmas)

Endocervix and high vaginal swabs were taken from the women under study, then, each specimen was directly inoculated into the liquid phase of the MDCS, mixed up well and then tilted once or twice to cover the upper portion of the slant for a while prior to incubation. All inoculated media were incubated aerobically at 37C° and observed daily for colour change from red to yellow in the liquid phase after 24 hrs. Then isolated colonies appeared after that on the slanted solid phase. We added sheep erythrocytes (7%) to the solid phase of the MDCS in several trials to detect the blood haemolysis by *M.fermentans* and *U.urealyticum*. as well, the egg yolk suspension (15 ml) was added to the standard PPLO agar for detect the lipolytic ability of *M.fermentans.*

III. Results

In our knowledge this study performs the first isolation of *M.fermentans*,*M.genitalium* and *M.penetrans* in Iraq with MDCS system and modified PPLO media according to the previous studies. Besides, it is the first isolation to both species: *M.hominis* and *U.urealyticum* by this method. The colonial growth was observed on the upper portion of the slant together with changing in colour of the liquid phase from red to yellow after 24 hrs. However, approximately 96 hrs. Period was necessary for full development of colonies on modified media of PPLO with MDCS for show the fried-egg appearance Figure 1, 2. Distribution of genital mycoplasmas and occupation is shown in Table (1), there was no statistically significant difference for M.hominis, M.fermentans, U.urealyticum, M.penetrans but a difference was found to be just significant for M.genitalium ($X2 = 11.333$; $P < 0.01$) when comparison between the two groups (housewife and employee). Concerning the usage of contraceptives (Table 2), a difference was found to be just significant for M.fermentans $P < 0.05$ and M.genitalium $P < 0.05$, in detection of genital mycoplasmas colonization in tested women under study that used and not used of

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contraceptive devices. A difference was found to be significant in the isolation of both *M.hominis* and *M.genitalium* from non-pregnant when compared with pregnant women. A significant difference was also noted in the isolation rates of: *M.hominis, M.fermentans* and *M.penetrans* from pregnant women at different gestational periods (trimesters) as shown in Table 3, 4.

A statistically significant difference at the level of ($P<0.01$) was noted in the isolation of both *U.urealyticum* and *M.genitalium* from endocervix region in comparison with high vaginal region as shown in Figure 3.Also, the bacteria (other than mycoplasmas) which isolated from women using contraception devices, they consisted of *S.aureus* as most frequently bacterial isolated in 20.0% followed by *K.pneumoniae*, *E.coli*, *P.aeruginosa* and *Proteus spp.* in (15.0%, 10.0%, 10.0%, 5.0%) respectively as shown in Table 5. Further more, the genital mycoplasmas were found as a single infection in 20 cases (16.9%) and mixed infection with other causative agents in 21 cases (17.5%)Table 6.

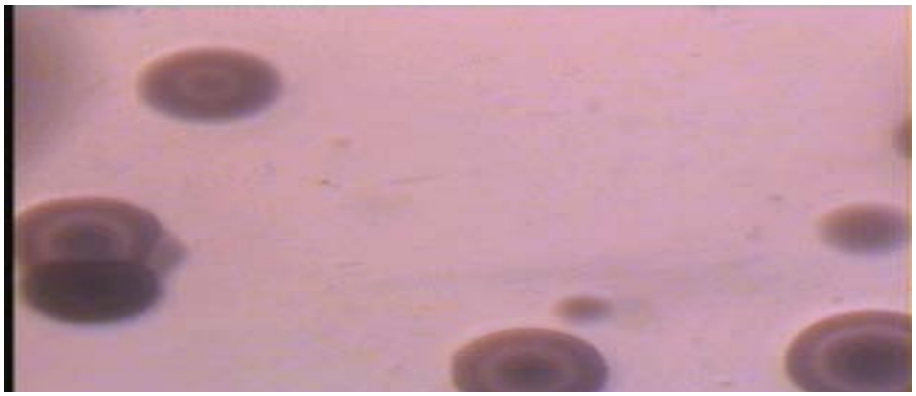


*Figure 1:*The fried egg colonies of *M.hominis*on modified PPLO medium (X 160)

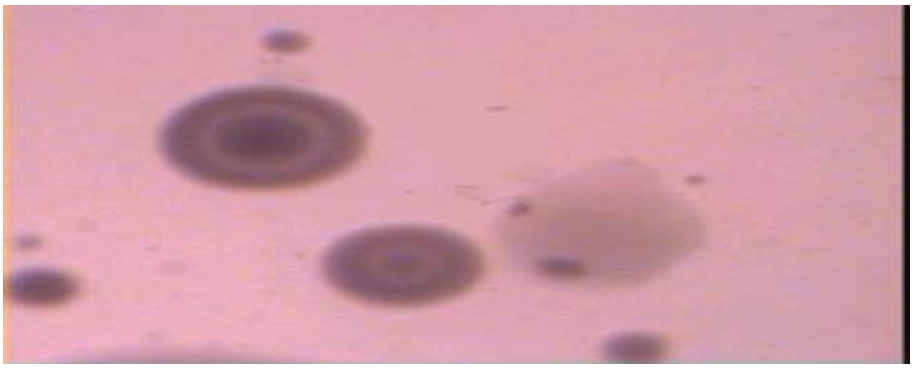


Figure 2: The fried egg colonies of *M.genitalium* on modified PPLO medium (X 160)

Table 1: Distribution of genital mycoplamas in relation to occupation

Occupation	No. of tested women	No. and (%) of women + ve in				
		M. hominis	*M.fermentans*	*U.urealyticum*	*M.genitalium*	*M.penetrans*
House wife	90	12 (13.3)	4 (4.4)	9 (10.0)	5 (5.5)	3 (3.3)
Employee	30	4 (13.3)	1 (3.3)	3 (10.0)	0 (0)	0 (0)
Total	120	16	5	12	5	3
X^2		0	0.286	0	11.333	3.000
P		NS	NS	NS	0.01	NS

P> 0.05 (NS)

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Table 2: Genital mycoplamas colonization correlated with usage of contraception

Women subjects	No. of tested women	No. and (%) of women + ve in				
		M. hominis	*M.fermentans*	*U.urealyticum*	*M.genitalium*	*M.penetrans*
Used	20	3 (15.5)	0 (0)	2 (10.0)	0 (0)	1 (5.0)
Not used	100	13 (13.0)	5 (5.0)	10 (10.0)	5 (5.0)	2 (2.0)
Total	120	16	5	12	5	3
X^2		0.143	6.250	0	6.250	1.286
P		NS	0.05	NS	0.05	NS

P> 0.05 (NS)

Table 3: Prevalence of Genital Mycoplamas in Pregnants Compared to Non-pregnant Women

Women subjects	No. tested	No. and (%) of women + ve in				
		M.hominis	*M.fermentans*	*U.urealyticum*	*M.genitalium*	*M.penetrans*
Pregnant	40	3 (7.5)	1 (2.5)	4 (10.0)	0 (0)	2(5.0)
Non-pregnant	50	8 (16.0)	2 (4.0)	5 (10.0)	2 (4.0)	1 (2.0)
X^2		12.040	0.346	0	4.000	2.25
P		0.01	NS	NS	0.05	NS

Table 4: Prevalence of Genital Mycoplamas in Pregnant Women at Different Gestational Periods

Pregnant women	No. tested	No. and (%) of women + ve in					Total of G.M (%)
		M.hominis	*M.fermentans*	*U.urealyticum*	*M.genitalium*	*M.penetrans*	
First trimester	10	1 (10.0)	1 (10.0)	1 (10.0)	0 (0)	1 (10.0)	4 (40.0)
Second trimester	10	0 (0)	0 (0)	1 (10.0)	0 (0)	0 (0)	1 (10.0)
Third trimester	20	2 (10.0)	0 (0)	2 (10.0)	0 (0)	1 (5.0)	5 (25.0)
X^2		10.110	23.333	0	0	10.000	34.000
P		0.01	0.01	NS	NS	0.01	0.01

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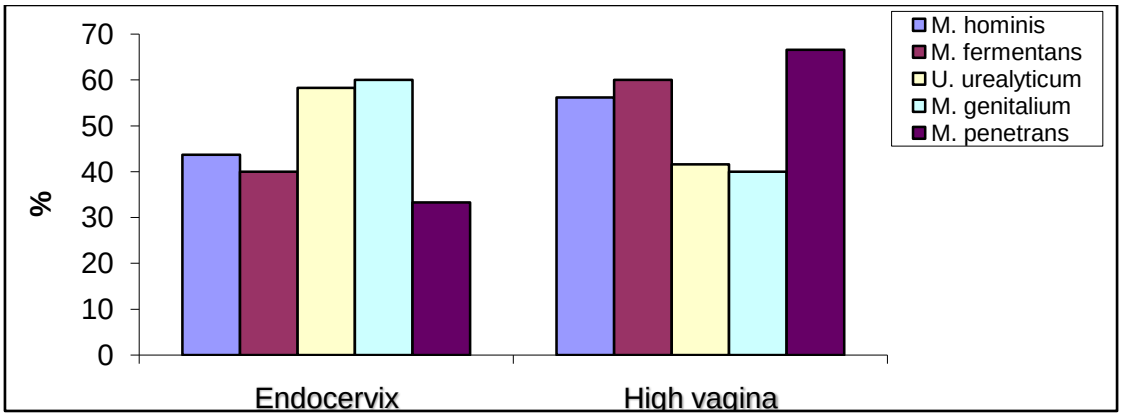


Figure 3: Correlation between genital mycoplasmas and sources of isolation

Table 5: Bacteria which isolated from women using contraception (20 women).

Bacteria	No. and (%) of infected women + ve in
Staphylococcus aureus	4 (20.0)
Klebsiella pneumoniae	3 (15.0)
Escherichia coli	2 (10.0)
Pseudomonas areuginosa	2 (10.0)
Proteus spp.	1 (5.0)
Total	12 (60.0)
X2	6.84
P	0.01

Table 6: Presence of genital mycoplasmas alone or in conjunction with other bacteria

Total of genital mycoplasmas	Alone	In conjunction with				
		S.aureus	*K.pneumoniae*	*P.aeruginosa*	*E.coli*	*Proteus. spp*
41	20	7	5	4	3	2

IV. Discussion

Prevalence of mycoplasmas in the female genital tract depends on numerous factors such as age, level of socioeconomic status, sexually active, disorders in the menstruation, pregnancy, infertility, urogenital complaints and use of contraceptive device [19-21, 38-39]. According to our knowledge, this study was the third in Iraq to determine the prevalence of genital mycoplasmas in Iraqi women and the second in Basrah. Further, the current study shows for the first time the isolation and identification of: M.fermentans, M.genitalium and M.penetrans in Iraq besides the first isolation of both M.hominis and U.urealyticum by using modified PPLO medium and MDCS system for the primary isolation of genital mycoplasmas from clinical samples then, detection with biochemical test [18, Holt et a, 1994]. The modified PPLO medium consist of antimicrobial agents: such as thallium acetate and penicillin, the other supplements consist of glucose solution; sodium deoxyribonucleate (DNA calf thymus) solution; hydrogen phosphate solution and cresol red solution as an indicator of the mycoplasmal growth in addition to PPLO broth/agar media; yeast extract; sodium chloride and horse serum, this method offered multi merit symbolized by the less contamination as a result of elimination of transport media, fast of results appearance, supply a pliability in the kinds of media represented by liquid and solid phases, it is inexpensive because its consumed a small quantities of liquid and solid media furthermore, it has a short incubation time period within one test tube. It is the first technique used to determine the prevalence of genital mycoplasmas in women in Basrah City in comparison with studies of Simhairi (1990 and Al-Bahlis' (1993).

Table 1 shown, there was no statistically significant difference for M.hominis, M.fermentans , U.urealyticum, M.penetrans but a difference was found to be just significant for M.genitalium($P < 0.05$), when comparison between the two groups (housewife and employee) in related of occupation, this could be association with educational levels of women, awareness, culture and interest in personal hygiene [21, 26]. Concerning the usage of contraceptives (Table 2) which shows, a significant difference for M.fermentans($P < 0.05$) and M.genitalium ($P < 0.05$) in detection of genital mycoplasmas colonization when, tested women under study that used and not used of contraceptive devices, that is due to, the colonization of genital mycoplasmas in women is linked to younger age, contraceptive devices use, lower socioeconomic

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status, and sexual activity with multiple partners and other factors [19, 21]. A difference was found to be significant in the isolation of both *M.hominis* and *M.genitalium* from non-pregnant when compared with pregnant women. A significant difference was also noted in the isolation rates of: *M.hominis, M.fermentans* and *M.penetrans* from pregnant women at different gestational periods (trimesters) as shown in Table 3, 4. Simhairi (1990), Al-Bahli (1993) found no significant difference in the prevalence of M.hominis and U.urealyticum among pregnant women as compared to non pregnants, as well as, to gestational stage of pregnancy. Their results were somewhat consistent with the results of the study. Similar as well as contrary observations regarding the prevalence of M.hominis of other strains in pregnant women have been reported by many investigators (Csonkaetal., 1966; Jones, 1967; Harwicketal., 1970; Delouvoisetal., 1975; Taylor-Robinson and McCormack, 1979 and Iwaskaetal., 1986a). This controversy may be attributed to several factors like strain variations of the microorganisms, difficulty in determining a precise matching control with respect to sexual experience, especially in relation to the number of partners and population peculiarities, like differences in the socioeconomic status and hygienic standard (McCormack etal.,1973b). In present study we found,a statistically significant difference at the level of ($P<0.01$) in the isolation of both *U.urealyticum* and *M.genitalium* from endocervix region in comparison with high vaginal region (Figure3), the results ofpresent study in agreementswith R.M.Al-Mosawi, 2009. And in study of Upadhyayaetal. (1983) the frequency of the isolation of *U.urealyticum* was significantly higher in the infertile group than in a group of pregnant women. Thereupon, the results of the current study which show that both *U.urealyticum* and *M.genitalium* were recovered in a higher isolation rates from endocervix than high vagina of infected women may be associated with the inflammatory disease leading to infertility. Several investigators have demonstrated some kind of association between female infertility and *U.urealyticum* infection (Gnarpe and Friberg, 1973a; Fowlkersetal., 1975; Upadhyayaetal., 1983; Taylor-Robinson, 1986).

In present study, the bacteria (other than mycoplasmas) were isolated from women using contraceptions, they consisted of *S.aureus* as most frequently bacterial isolated in 20.0% followed by *K.pneumoniae*, *E.coli*, *P.aeruginosa* and *Proteus spp.* in (15.0%, 10.0%, 10.0%, 5.0%) respectively as shown in Table 5, those finding were in accordance with Al-Bahili, 1993 study.

Moreover, in the current study, the genital mycoplasmas were found as a single infection in 20 cases (16.9%) and mixed infection with other causative agents in 21 cases (17.5%), as showed in Table 6, these results were somewhat in agreement with Simhari, 1990; Al-Bahli, 1993; Al-Mosawi, 2009.

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Antibiotic Resistance in Uropathogenic *Citrobacter* Spp. Isolated from Internally Displaced Persons with Urinary Tract Infections in Internally Displaced Camps, Maiduguri

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Abstract- *Background:* We sought to investigate the health challenges attributed to urinary tract infections (UTI) amongst internally displaced persons (IDPs) in north- eastern Nigeria.

Methods: Urine specimens were collected, micro- biologically processed and subjected to antimicrobial susceptibility testing using standard agar disc diffusion techniques in accordance with standard protocols.

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Keywords: citrobacter, uropathogenic, antimicrobial susceptibility, internally displaced persons, multi-drug resistance, urinary tract infections.

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Abdulrasheed B. Abdu [α], Mohammed Kachallah [σ], Kemebradikumo D. Pondei [ρ] & Adebayo Lamikanra [ω]

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Conclusion: The results of this study suggest that there is a need for continuous investigation of the health needs of IDPs who are particularly vulnerable to various health challenges in order that they can be provided with adequate and comprehensive healthcare services.

Keywords: citrobacter, uropathogenic, antimicrobial susceptibility, internally displaced persons, multi-drug resistance, urinary tract infections.

I. Introduction

Internally displaced persons (IDPs) are 'persons or groups of people who have been compelled to flee or leave their homes or places of customary residence, in particular as a result of, or in order to avoid the effects of armed conflicts, situations of generalised violence, violations of human rights or natural or man-made disasters, and who have not crossed an internationally recognised state border [1]. Controversially, IDPs are often referred to as refugees, even though they do not fall within the legal definitions of being called refugee because, they are distinct from refugees who are displaced outside their national borders [2, 3].

Estimates from the Internal Displacement Monitoring Centre (IDMC) indicate that the number of people displaced annually by conflict and violence has increased globally since 2003[4]. A massive 40.3 million of them were newly uprooted during 2016 equalling to 15,000 people displaced every day in African countries alone [4, 5, 6, 7, 8]. By the end of 2017, a record-breaking 65.6 million people had become displaced within their own country as a result of violence [5].

Three quarters of these IDPs reside in ten countries of the world, and five of these are located in Sub Saharan Africa. The total number of people displaced by conflict in the region is almost 12 million [4, 6].The IDMC's Global Overview [6] reported that the majority of the increase in new displacement during 2015 was the result of protracted crises in the Democratic Republic of the Congo, Iraq, Nigeria, South Sudan and Syria. In total, these five countries accounted for 60 per cent of new displacement worldwide [6].

In Central Africa, conflict and violence have resulted in over a million displacements of people in the Democratic Republic of Congo [4]. Other African countries which have had large numbers of IDPs in the past decade are Somalia, Uganda, Kenya and Sudan [9].

In Nigeria, the insurgent activities of Jamā'at Ahl as-Sunnah lid-Da'wah wa'l-Jihād (Islamic State's West Africa Province) commonly called Boko Haram (BH) in the past decade have forced more than 2,152,000 people to flee their homes with 1,434,142 of these coming from Borno State [10]. This has resulted in an unprecedented humanitarian crisis in the North eastern part of the country and the Lake Chad region [4]. Inter communal clashes resulting from ethno religious disputes, between Fulani herdsmen militia and farmers

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have also resulted in over 700,000 people being displaced from the Middle Belt region of Nigeria [4].

Internal displacement has significant effects on the health and well-being of the affected populations. These impacts could be categorised as directly due to violence and injury or indirectly due to increased rates of communicable diseases and malnutrition [11, 12, 13, 14]. According to Owaje *et al*. [15] there areseveral risk factors, working in synergy during displacement which promote communicable diseases. These factors include the massive movement of populations and resettlement in temporary locations, overcrowding, economic, environmental degradation, poverty, inadequate availability of potable water, poor sanitation and bad waste management [11]. These conditions are further complicated by the absence of shelter, food shortages and poor access to healthcare [16]. In Sub-Saharan Africa, the combined effects of these factors depend on the location and increased risk of diseases such as acute respiratory infections [17], diarrhoeal diseases [18] and scabies [19].

Diarrhoeal and Urinary tract diseases are major causes of morbidity and mortality among IDPs and mainly result from substandard or inadequate sanitation facilities, poor hygiene and poor hand washing practices due to scarcity of soap and water [16].

Urinary Tract Infection (UTI) continues to be one of the most important causes of morbidity and mortality. Hitherto, UTIs caused by *Citrobacter* species have been described in 5 to 12% of bacterial urine isolates in adults [20, 21].The genus *Citrobacter* is a distinct group of aerobic, Gram negative bacilli from the *Enterobacteriaceae* family, widely distributed in water, soil, food and intestinal tract of humans and animals. We report here the emergence of *Citrobacter* as a significant uropathogen among IDPs living in IDP camps in Maiduguri, Nigeria, and their susceptibilities to antimicrobial agents in order to generate data that will improve the efficacy of the treatment of this infection.

II. Materials and Methods

The study was conducted between February 2017 through January 2018 and the studied population was composed of 5000 IDP patients seeking medical attention at out-patient IDP-clinics in Maiduguri (Muna Garage, NEMA mobile Clinics, UNICEF Clinic, Jidari, ALIMA Clinics, Arabic Teachers College, Teachers Village, NYSC Camp, Gubio) metropolis. The benchmarks for patient inclusion were -patients who presented with UTI symptoms: like burning during micturition, fever, pyuria, frequency of urine, dysuria, haematuria, flank pain, suprapubic discomfort, and whose urine specimens showed significant bacterial growth ($\geq 10^5$ CFU/mL) associated with a white blood cell count of $>10^4$/mL as outlined by Metri and Jyothi [22].

a) Specimen Collection

Informed verbal consent was obtained from all patients prior to specimen collection. Afterward, they were educatedon the clean-catch midstream urine techniques as documented by Collee *et al*. [23] and Ochada *et al*. [24]to collect urine specimens of at least 20mL into a sterile Universal container (Sterling, UK). For female patients, after proper positioning of the thigh, they were instructed to spread the labia and clean the area with sterile swabs, then pass a small amount of urine into the toilet, and finally urinate into the container. For male patients, after hand washing, a clean-catch midstream urine sample was collected after cleaning of the glans with sterile swabs. The specimens were labelled appropriately, transported to the laboratory, and stored at 4°C for further analyses.

b) Specimen Processing, Identification and Maintenance

In the laboratory, a calibrated loop method was used for the isolation of bacterial pathogens from urinary specimens. A sterile 4.0 mm platinum wired calibrated loop was used to deliver 0.001mL of urine. Concurrently, a loopful of urine sample was plated on Cystine-Lactose-Electrolyte Deficient (CLED) agar, Mannitol Salt (MSA) agar, MacConkey agar, and blood agar medium (Biotech Laboratories Ltd. UK). The inoculated plates were incubated aerobically at 37°C for 24 h and in cases where no growth was observed for 48 h. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. By the description of Prakash and Saxena [25], a urine specimen was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$ cfu/mL or when an organism was cultured at a concentration of 10^4 cfu/mL and >5 pus cells per high-power field, epithelial cells, casts, and crystals were observed on microscopic examination. Identification of bacterial isolates to species level was done on the basis of their cultural characteristics as illustrated by Murray *et al*. [26] and standard biochemical characteristics was conducted on API 20E (Biomerieux, France). Confirmation of isolates as *Citrobacter* spp., was done using Polymerase Chain Reaction (PCR) as described by Thepa and Tribuddharat [27]. Identified and pure isolates were cryopreserved at -84°C.

c) Antibiotic Susceptibility Testing

The antimicrobial susceptibility pattern of all the isolates were tested by employing the modified single disc diffusion technique described by the Clinical and Laboratory Standards Institute (CLSI, 2017) [28]. The antibiotics tested were Amikacin (10µg), Amoxicillin (25µg), Amoxicillin/clavulanic acid (30µg), Ceftriaxone (30µg), Cephalexin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25µg), Erythromycin (15µg), Gentamycin (10µg), Levofloxacin (5µg), Nalidixic acid (30µg), Nitrofurantoin (300µg),

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Norfloxacin (5µg), Ofloxacin (5µg), Perloxacin (5µg), Streptomycin (10µg) and Tetracycline (30µg), all obtained from Oxoid (England). Breakpoints and interpretation for susceptibility/resistance was based on CLSI [28] criteria. Standard strains of *E. coli* ATCC25922, and *S. aureus* ATCC25923 were used routinely in this study as control organisms. We defined any isolate as multidrug resistant (MDR) strain if it shows resistance against three or more different antibiotics (29).

Resistance against different antibiotics appears on the same bacterial strains more often than expected.

d) Statistical Analysis

Statistical analysis was done using SPSS (version 20) to determine frequency distribution, mean, harmonic mean, standard deviation, analysis of variance (ANOVA), Duncan Multiple Range and Pearson correlation coefficient.

i. *Ethics*

Ethical approval was secured from Research Ethics Committee of the University of Maiduguri Teaching Hospital. Permission from Camp Clinical Directors was also obtained.

III. Results

In order to categorise symptomatic urinary tract infections among the IDPs, 5000 mid-stream urine specimens were collected, processed and the results analysed. Of the 5000 urine specimens collected 4300 (86.00%) were found to be positive for significant bacteriuria while 700 (14.00%) yielded no growth. Among these 4300 culture positive specimens, 4688 (i.e. 1.09 isolates per sample) uropathogenic bacteria isolates were obtained, of which 4110 had a single pathogen and 578 had two types of bacteria isolates. The age of our patients ranged from 1 to 72 years, with a mean of 34.2 ± 12.6 years and a median of 37 years. UTI was significantly more prevalent among the females (p value = 0.002) than the males with 3474 (80.79%) significant specimens obtained from females while 826 (19.21%) were from the males, thus making male: female ratio of 1:4.2.

As presented in Figure 1, overall, Gram-negative bacteria accounted for 83.8% of the isolated uropathogens, while Gram positive bacteria accounted for 16.2%. *Citrobacter* species accounting for 1407 (30.01%) of all the isolates were found to be second most common uropathogens among the IDPs following *Escherichia coli* with 1896 (40.44%) while *Enterobacter aerogenes* (presently known as *Klebsiella aerogenes*) was the least isolated bacteria with 57 (1.22%).

Table1 shows that the number of uropathogenic *Citrobacter* isolated from females were significantly higher than those from their male counterparts ($p<0.05$) with 1182 (84.0%) from the females while 225 (16.0%) were from the male.

Figure 2 shows Age-wise distribution of uropathogenic *Citrobacter* spp. isolated. As shown in all age groups, the isolation of *Citrobacter* species from the urine of the subjects increased with age and picked in the 31 to 40 years age group and then declining to its lowest level in the 51 to 60 years age group before rising again.

Figure 3, depicts the *in vitro* susceptibility patterns of the isolated *Citrobacter* spp. to eighteen different antimicrobial agents. As illustrated, all the uropathogenic *Citrobacter* isolates were resistant to Amoxicillin, Cephalexin, Co-trimoxazole, and Tetracycline. While, more than 50% of the isolates showed resistance to Amoxicillin/clavulanic acid (98%), Ceftriaxone (90%), Erythromycin (85%), and Ciprofloxacin (56%). In descending order, resistance was shown to Chloramphenicol and Levofloxacin (46%) each, Pefloxacin (44%), Norfloxacin and Ofloxacin (43%) each, Streptomycin (32%), Gentamicin and Nalidixic acid (10%) each, Nitrofurantoin (6%) while none of the isolates showed resistance to Amikacin (0%).

Table 2 shows the frequency of *Citrobacter* spp., isolates and their antibiotic resistance patterns. The result showed that *Citrobacter freundii* (850 isolates, 60.4%) was the most predominant among the uropathogenic Citrobacter species encountered in this study. This was followed by *C. koseri* (421 isolates, 29.9%), while *C. amalonaticus* and *C. intermedius* accounted for 68 (4.85%) isolates each.Additionally, all the isolated *Citrobacter* were multidrug resistant (i.e. showed resistance to at least three classes of the tested antimicrobial agents).

IV. Discussion

In spite of the multitudinous health difficulties confronted by the IDPs, there is limited documentation of these health challenges. Emphasis has been bestowed more on their physical and mental health challenges [30, 31, 32] which for example includes sexual assaults and substance abuse [33, 34]. However, little or no reports are available about their urogenital challenges, hence the significance of this present study. We investigated the prevalence and contribution of UTIs, particularly those attributable to uropathogenic *Citrobacter* among these susceptible groups of individuals. From this study, the prevalence of UTI among the IDPs presenting with urinary symptoms is 86.0%, while the prevalence rate accountable to uropathogenic *Citrobacter* is 30.01%. With regards to prevalence of uropathogens among IDPs, there is no baseline data for reference. Nevertheless, this high rate of UTI prevalence observed is consistent with previous report of 75.0% and 80.0% recorded in the same Maiduguri area amongst patients seeking medical attention by Kachalla *et al.* [35] and Abdu *et al*. [36] respectively. This high isolation rate had been attributed

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to various reasons such as the differences in specimens, specimen collection and processing methods [37]. Furthermore, this high prevalence rates could be due to environmental factors in the IDP-camps including poor waste disposal and environmental sanitation, overcrowding, inadequate access to water supply and healthcare services as identified by Lam *et al*. [38]

In humans, the emergence of *Citrobacter* in a wide spectrum of infections such as in the urinary tract, respiratory tract, wounds, bone, peritoneum, endocardium, meninges and blood stream is on the increase [39, 40, 41, 42]. Among these various sites of infection, the urinary tract is regarded as the most common [43, 44], with isolation rate ranging from 5 to 44% [44, 45, 46, 47]. This is in comparison with 30.01% observed in this study. *Citrobacter freundii* (60.4%) was found to be the most prevalent among the uropathogenic *Citrobacter* species. While *Citrobacter koseri* constitute 29.9%, *Citrobacter amalonaticus* and *Citrobacter intermedius* constituted 4.85% of the isolates each. However, the frequency of *Citrobacter* in urine specimens varies from one study to the other [37, 45, 47, 48]. In the present study, women have higher rate of uropathogenic *Citrobacter* than men (Table 1), because anatomically, in females, the urethra has been known to be shorter and closer to the anus [49]. Other investigators have also reported similar findings to ours [37, 50, 51]. Furthermore, the high prevalence of uropathogenic *Citrobacter* among these female groups aside from sexual activities, may be related to the study participants whose immune system might have been impaired. Nonetheless, study conducted in India has shown sharp contrast to our findings where the condition was more prevalent in males when compared to females counterparts [22].

Globally, there is an increasing incidence of resistance among uropathogens to older antimicrobial agents and also to the newer and supposedly more potent antimicrobial agents [52]. The *in vitro* antibiotic susceptibility profile of the uropathogenic *Citrobacter* species isolated in this study showed a discouraging pattern with multidrug resistance being prominent among the organisms against which the drugs were tested. Majority of the isolates in the current study were found to be resistant to Amoxicillin, Amoxicillin/ clavulanic acid, Ceftriaxone, Cephalexin, Ciprofloxacin, Co-trimoxazole, Erythromycin, and Tetracycline [Figure 3]. This has important implications as most patients in our locality receive these drugs, or a combination of these drugs as empirical therapy or as definitive treatment.

As revealed by the present study none of the isolates was resistant to Amikacin, while the values of 10% and 32% of the uropathogenic *Citrobacter* isolates were resistant to Gentamicin and Streptomycin respectively. Therefore the aminoglycosides should be considered as being the most effective antimicrobial drugs of choice for treating uropathogenic *Citrobacter* infections and should be administered while awaiting the culture result. This outcome is similar to previous studies [36, 53, 54, 55, 56]. Earlier, Abdu and Lamikanra [57] suggested that what was responsible for the high susceptibility recorded to the aminoglycosides and one of such explanations was the fact that aminoglycosides are rarely abused as they are administered parenterally, a dosage form which is far less liable to self- medication than the orally administered antibiotics in this locality, furthermore, the cost of Amikacin is about $70 per vial, taking it far beyond the reach of the vast majority of people in a locality where people are considered poor. Despite the impressive efficacy associated with the aminoglycosides, many studies have documented a contrary result with higher resistance to these agents among uropathogenic *Citrobacter* [22, 44, 45, 58]. Apart from their innate ability to transfer their resistance to aminoglycosides, one of the reasons suggested for the low efficacy of aminoglycosides in those studies was that they are frequently prescribed for treatment of infections [44].

With the increasing incidence of drug resistant organisms seen presently, there is need to evaluate the activity of Nitrofurantoin even though it is a drug such extensively drug-resistant strains. Yet, as revealed, Nitrofurantoin is the second most efficacious antimicrobial agent to the isolated uropathogenic *Citrobacter*. The maximum resistance percent value was found as 6% (94.0% susceptible), 42 isolates each for *C. freundii* and *C. koseri* (Table 2). Since good *in vitro* activity was shown by Nitrofurantoin it may be considered as first line oral therapy for IDPs patients with UTI. There is very limited data on Nitrofurantoin activity against *Citrobacter* isolates. Nevertheless, the few available reports were found to be similar to the present findings [53, 59]. Various reports have also corroborated our findings with Nitrofurantoin susceptibility among uropathogenic *Escherichia coli* [60, 61, 62, 63]. Besides its multiple mechanisms of action that have enabled it to retain potent activity against pathogens [60, 64], other possible explanations that might have allowed Nitrofurantoin to still show good *in vitro* efficacy against uropathogenic *Citrobacter* in this study might be attributed to its unpleasant side effects such as, gastrointestinal discomfort, pulmonary, liver, and nerve toxicity [65, 66] that discourage its abuse leading from extensive self-medication. However, in contrast to the efficacious outcome of Nitrofurantoin reported in this study, a significant increase in resistance of uropathogenic *Citrobacter* and uropathogenic *Escherichia coli* to Nitrofurantoin have been reported [67, 68, 69, 70].

In this study, the overall susceptibility of the uropathogenic *Citrobacter* isolated from the IDPs for the fluoroquinolones group was worrisome. Surprisingly,

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apart from the Nalidixic acid that the isolates showed least resistance to (10%), resistance to other groups were significantly high. As revealed by the study, Ciprofloxacin resistance was found to be most frequently encountered with 56%, this is followed by Levofloxacin with 46%. Perfloxacin was next with 44%, while the value obtained for both Norfloxacin and Ofloxacin was 43%. This pattern of resistance is in agreement with the results of previous studies [22, 37, 45, 71], an outcome suggesting that the fluoroquinolones have limited usefulness in the management of uropathogenic *Citrobacter* within the studied environment. This is unexpected considering that the fluoroquinolone group of antimicrobial agents are employed as empirical therapy or as definitive treatment for UTI and were incorporated into the therapeutic management of infectious agents only recently [57]. Furthermore, the ability of these organisms to spread easily from person to person with consequential remedial complications having recognised them as efficacious anti-infective drugs only a few years ago [72]. This outcome thus calls for urgent and drastic regulatory measures in order to combat this situation, for failure to do so, we may be trending towards a post-antibiotic era which calls for a great deal of research into the development of new antibiotics. Studies have also shown that mutations in *gyrA* and *parC* genes are the most common mechanism involved in high-level quinolone resistance, in addition to the spread of plasmid- mediated quinolone resistance genes and efflux-pump mutants [73].

In spite of the fact that Chloramphenicol is strictly regulated and it is not commonly prescribed due to its adverse effect (aplastic anaemia), moderate rates of resistance observed in the present study (46%) may be due to the fact that Chloramphenicol is widely used in our study environment with other broad spectrum antibiotics in the treatment of life threatening infections. The result obtained in this study is in agreement with a study in Ethiopia which documented Chloramphenicol resistant strains of *Citrobacter* spp. from UTI [37, 48]. Nevertheless, it is not in agreement with the study of Liu *et al*. (2017) who reported a high susceptibility rate of Chloramphenicol (87%) in China [56].

In the current study, all the uropathogenic *Citrobacter* isolates were resistant to Co-trimoxazole and Tetracycline, while 85% were resistant to Erythromycin. These findings authenticate the figures from previous studies [37, 71]. However, unlike in these previous studies, these isolates showed highest rates of resistance to Co-trimoxazole, Erythromycin and Tetracycline. Highest resistance (100%) against 18%; 26%; and 32% reported for Co-trimoxazole by Metri *et al*. [45], Liu *et al*. [56] and Mishra *et al*. [53] respectively. For Erythromycin the high resistance rate of 85% was reported against 3.28% Azithromycin by Liu *et al* [56].

The percent resistance values of the β-lactam group were similar (Table 2). Among the β -lactam antibiotics, Amoxicillin and Cephalexin showed the highest percent (100%) resistant isolates; the resistance pattern slightly decreased in the following order: Amoxy-clav (98%) and Ceftriaxone (90%). As illustrated, there is no significance difference between the patterns of resistance shown by the uropathogenic *Citrobacter* to Amoxy-clav and Ceftriaxone. For Amoxy-clav: Ceftriaxone, 100%:100% of *C. freundii* isolates had the highest percentage of resistance, while the values of resistance for *C. koseri*, *C. amalonaticus* and *C. intermedius* were 100%:95%; 79.4%:11.8%; and 79.4%:11.8% respectively.

This increased resistant level may be ascribed to the easy access to these drugs in our study environment. Furthermore, these drugs are purchased directly over-the-counter from pharmacies and other unauthorised sources without a doctor's prescription and are commonly used for a broad spectrum of infections. With this pattern of resistance, it is recommended that most *β-lactams* antibiotics should not be used as first line agents in the blind treatment of UTIs. This is more so, as ascribed by Paramythiotou and Routsi, [74] infections caused by resistant pathogens are associated with higher rates of morbidity and mortality than infections caused by susceptible pathogens.

Multiple antibiotic resistance (MAR) index reveals (table not shown) that all of the isolates were resistant to at least three antibiotic groups This highlighted the fact that, most of the antibiotics tested in this study have lost their potency in respect of the organisms against which they are deployed. This could be linked to several factors including: possession of multiple resistance genes in the bacterial genome that enable them to transfer resistances to virtually all the antibiotics, source of the isolates, its ability to evade antibiotic effects and variation in antibiotic concentration. Many studies have identified bacterial source as an important determinant of MAR especially due to *Citrobacter* spp. when it occurred in an infection. This emergence has coincided with previous findings that documented *Citrobacter* spp. is often resistant to multiple classes of antibiotics, suggesting that both clinical and environmental strains may be a reservoir of antimicrobial resistance determinants [39, 56, 75, 76, 77, 78]. Many factors contribute to the emergence of MAR in *Citrobacter* among which over prescribing of antibiotics by clinicians, over-usage and incomplete course of antibiotics by patients, availability of the antibiotics could not be ignored in regions like ours. Additionally, environmental and personal hygiene can also contribute to the spread of resistant species among people especially in clinical settings. Mass media campaigns, regular training, and reformation of drug policies would to a significant extent alleviate the

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increased spread of MAR isolates among the populace. The findings have revealed that there is a crucial necessity for persistent monitoring of susceptibility of pathogens in different populations to commonly used anti-microbial agents. The data obtained from this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics.

V. Conclusion

In conclusion, the study highlights the emergence of *Citrobacter* spp., a rare bacterium as the second most common urinary pathogen, which is multidrug resistant among the IDPs. UTI particularly those associated by Multidrug resistant organisms (e.g. *Citrobacter* spp.) should be incorporated as one of the innumerable health challenges encountered by Internal Displacement and remain a pressing issue. A great deal therefore remains to be done to address IDPs prevention and control to decrease UTI especially those associated with *Citrobacter* morbidity and mortality. This protection and assistance needs to continuously evaluating susceptibility pattern of uropathogens to traditional as well as new antimicrobials in well-defined populations and limiting the inappropriate and injudicious use of antibiotics so as to prevent further emergence of drug resistance, to find enduring solutions to their plight and to prevent further displacement from taking place. However, this requires intervention of different agencies, government and non-governmental bodies.

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Conflicts of Interest
None.

Author's Contributions
Abdulrasheed Abdu: Conception and design of the study, drafting and review of article, contributing to intellectual context.

Mohammed Kachallah: Data collection analysis and literature survey.

Kemebradikumo D. Pondei: Contributing to intellectual context.

Adebayo Lamikanra: Contributing to intellectual context and final review of manuscript.

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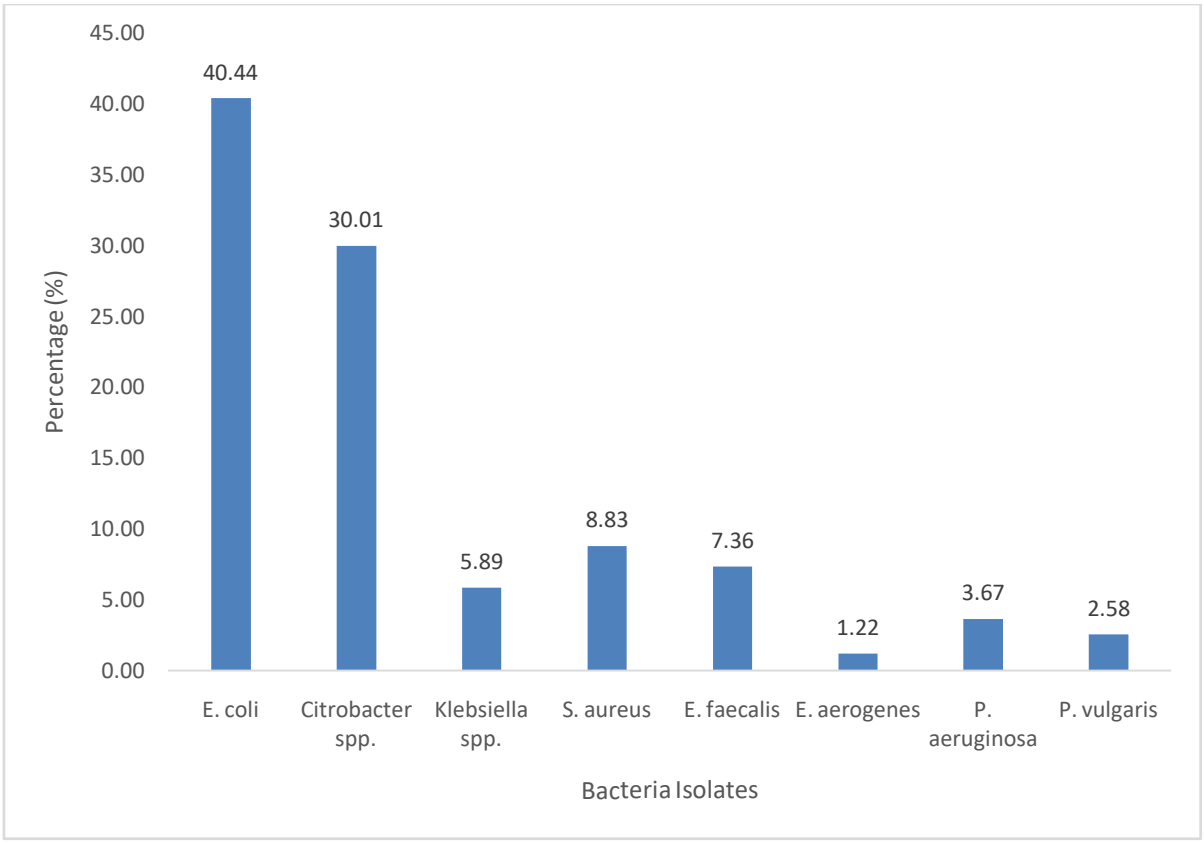


Figure 1: Frequency of *Uropathogenic* Bacteria isolated from Internally Displaced Persons with UTI in IDP Camps, Maiduguri, Nigeria, February 2017 through January 2018.

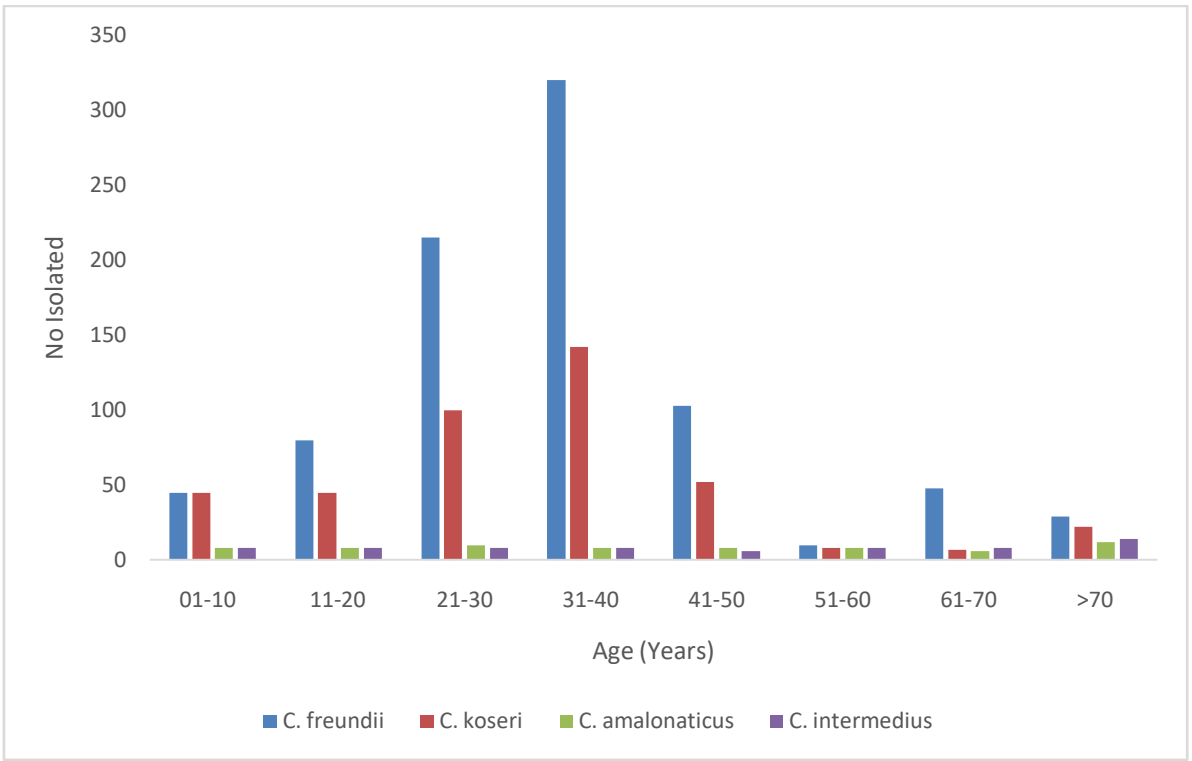


Figure 2: Prevalence of *Uropathogenic Citrobacter* spp. by Age Distribution from Internally Displaced Persons with UTI in IDP Camps, Maiduguri, Nigeria, February 2017 through January 2018.

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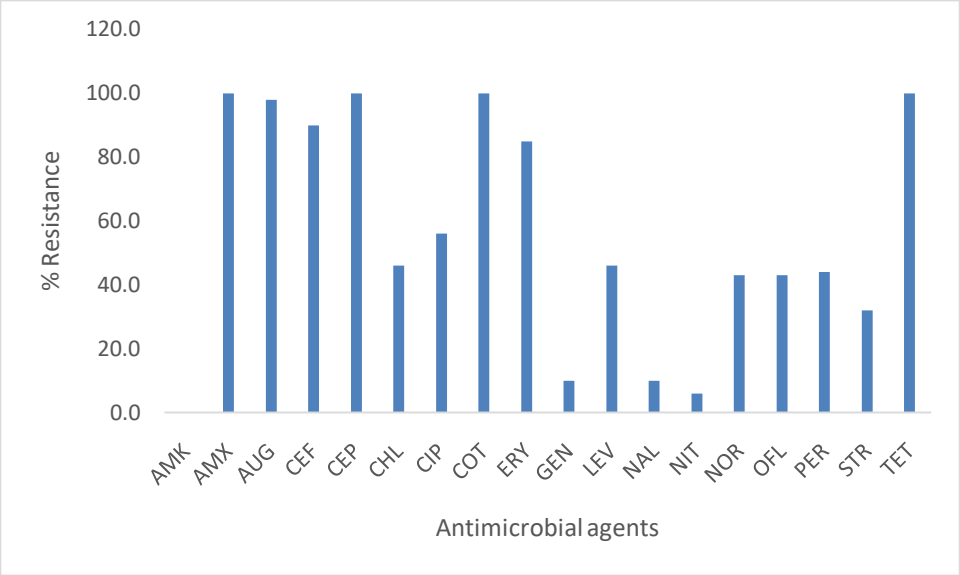


Figure 3: Antibiotic resistance patterns of *Uropathogenic Citrobacter* spp., from Internally Displaced Persons with UTI in IDP camps, Maiduguri, Nigeria.

KEY

AMK-Amikacin, AMX- Amoxicillin, AUG-Amoxicillin/clavulanic acid,CEF-Ceftriaxone, CEP- Cephalexin, CHL-Chloramphenicol, CIP-Ciprofloxacin, COT- Co-trimoxazole, ERY-Erythromycin, GEN-Gentamycin, LEV-Levofloxacin, NAL- Nalidixic acid, NOR-Norfloxacin, NIT – Nitrofurantoin, OFL- Ofloxacin, PEL- Pefloxacin, STR- Streptomycin and TET-Tetracycline.

Table 1: Age and sex distribution of Internally Displace Persons with uropathogenic *Citrobacter* spp. infection in Internally Displaced Camps, Maiduguri, Nigeria

Age (Years)	Male	Female	Total (%)
0 - 10	25	81	106(7.5)
11 - 20	20	121	141(10)
21- 30	30	303	333(23.7)
31 - 40	50	428	478(34)
41 - 50	35	134	169(12)
51 - 60	20	14	34(2.4)
61 – 70	30	39	69(4.9)
>70	15	62	77(5.5)
Total	225	1822	1407(100)

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Table 2: Percentage of Resistance by species of *Uropathogenic Citrobacter* isolatesfrom Internally Displaced Persons with UTI in IDP camps, Maiduguri, Nigeria, February 2017 through January, 2018

Antibiotic	*Citrobacter* Isolates				TOTAL (%)
	C. freundii (850)	*C. koseri* (421)	*C. amalonaticus* (68)	*C. intermedius* (68)	
Aminoglycosides					
Amikacin	0	0	0	0	0
Gentamicin	11.3	8.3	7.4	7.4	141(10)
Streptomycin	38.8	23.8	14.7	14.7	450(32)
ß-lactam					
Amoxicillin	100.0	100.0	100.0	100.0	1407(100)
Amoxicillin/clavulanic acid	100.0	100.0	79.4	79.4	1379(98)
Ceftriaxone	100.0	95.0	11.8	11.8	1266(90)
Cephalexin	100.0	100.0	100.0	100.0	1407(100)
Quinolones					
Ciprofloxacin	72.9	33.3	20,6	20.6	788(56)
Nalidixic acid	11.3	8.3	7.4	7.4	141(10)
Levofloxacin	49.5	47.5	19.1	10.1	647(46)
Norfloxacin	45.9	47.3	11.8	11.8	605(43)
Ofloxacin	45.9	47.3	11.8	11.8	605(43)
Perfloxacin	46.0	47.5	20.6	20.6	619(44)
Macrolide					
Erythromycin	100.0	71.3	33.8	33.8	1196(85)
Phenicol					
Chloramphenicol	49.5	47.5	19.1	19.1	647(46)
Miscellaneous					
Co-trimoxazole	100.0	100.0	100.0	100.0	1407(100)
Nitrofurantoin	4.9	10.0	0	0	84(6)
Tetracycline	100.0	100.0	100.0	100.0	1407(100)

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Prevalence and Potential Risk Factors of Hepatitis B Virus in a Sample of Children in Two Selected Areas in Yemen

By Ahmed Mohsen Al-kadassy, Hassan A. Al-Shamahy, Bushra Mohammed Jaadan, Anwar G Al-Madhaji, Basam Basim Mohammed Al-Fraji, Mohammed Auad Ajrah & Manal Mutaher Ali Al- Hajj

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Abstract- The global epidemic of hepatitis B is a significant public health problem. The endemicity of HBV infection used to be believed high in Yemen. Data for prevalence of HBsAg among children in rural and urban areas in Yemen is scarce and incompetent. The study was made to determine the prevalence of HB surface antigen among children in 2 selected areas in Yemen. Eight hundred ad forty and 212 children were randomly selected from Sana'a city and Shabowah governorate respectively. Sera were tested for HBs antigen by ELISA technique and HB genome was tested for positive HB surface antigen specimens to confirm positivity using polymerase chain reaction (PCR) -based test. Each individual's data was collected in a pre-designed questionnaire including: sex, age and risk factors of HBV and prior vaccine of HBV.

Keywords: *hepatitis B virus, epidemiology, children, risk factors, yemen.*

GJMR-C Classification: *NLMC Code: QW 170*



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Prevalence and Potential Risk Factors of Hepatitis B Virus in a Sample of Children in Two Selected Areas in Yemen

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Abstract- The global epidemic of hepatitis B is a significant public health problem. The endemicity of HBV infection used to be believed high in Yemen. Data for prevalence of HBsAg among children in rural and urban areas in Yemen is scarce and incompetent. The study was made to determine the prevalence of HB surface antigen among children in 2 selected areas in Yemen. Eight hundred ad forty and 212 children were randomly selected from Sana'a city and Shabowah governorate respectively. Sera were tested for HBs antigen by ELISA technique and HB genome was tested for positive HB surface antigen specimens to confirm positivity using polymerase chain reaction (PCR) -based test. Each individual's data was collected in a pre-designed questionnaire including: sex, age and risk factors of HBV and prior vaccine of HBV.

The prevalence of HB surface antigen among children in Sana'a city was only 1.8%, and in Shabowah governorate was 3.8%. There was a significant association of non-vaccinated children, birth by cesarean, and with history of parental exposure with contracting HBV infection. Evidence from these studies in Yemen suggests that there is a steady increase in exposure to HBV over a lifetime. Hospital-acquired HBV infection is very common in Yemen, and high vaccination coverage rate should be achieved particularly in rural areas, in parallel with health education.

Keywords: hepatitis B virus, epidemiology, children, risk factors, yemen.

I. Introduction

Hepatitis B virus (HBV) infection is an important global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBV infection. The 10th leading cause of death worldwide, HBV infections result in 500 000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma; the last accounts for 320 000 deaths per year[1, 2, 3]. In developed countries, the disease is relatively rare and gained primarily in adulthood in which injection drug abuse and unprotected sex are the primary methods, whereas in Asia and most of Africa including Yemen, chronic HBV infection is common and usually acquired perinatally or in childhood[4-8]. The endemicity of infection was considered high in Yemen, where prevalence of positive HBsAg among adult's ranges from 8 % to 20 %, among infants was 4.1%, and up to 50 % of the populations generally have serological evidence of previous HBV infection in old reports[9-15]. However, recent studies reported a lower rate of HBsAg in which it ranges from 0.7-2% among general population including children [16, 17, 18]. More efficacious treatments, mass immunization programs, and safe injection techniques are essential for eliminating HBV infection and reducing global HBV-related morbidity and mortality [19]. Safe and effective vaccines against HBV infection have been available since 1982. The implementations of mass immunization programs, which have been recommended by the World Health Organization since 1991, have dramatically decreased the incidence of HBV infection among infants, children, and adolescents in many countries [1, 2]. However, not all countries have adopted these recommendations and there remains a large number of persons that were infected with HBV which including Yemen in which the coverage rate of HBV vaccine in urban was only 69.9%, [3, 17]. The main aim of this study is determine the prevalence of HB surface antigen among sample of children in 2 selected areas in Yemen and analysis potential risk factors of HBV transmission among the selected children.

II. Subjects and Methods

a) Study area

This cross-sectional sero-epidemiological study was conducted in healthy children less than 11 years of age in Sana'a city and in healthy children less than 16 years of age in Shabwah governorate Yemen. The Yemen is located on the Arabian Peninsula in Southwest Asia. It is bordered by Saudi Arabia to the north, the Red Sea to the west, the Sultanate of Oman to the east and the Arabian Sea to the south. The

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population in 2008 was estimated at 21,843,554, living in 3,058,299 households. Population structure is typical of a developing country, with the rural population comprising about 71% of the total population. The majority of the population is young, with nearly half (45%) below age 15 years, while the elderly age group (over 64 years) represents only about 3.4%. The literacy rate is about 47% among those 15 years and older (males 63%, females 31%), the total fertility rate 6.2, the average household size 7.1 persons, the poverty rate about 47%, and the annual growth rate of population 2.9%. Life expectancy at birth male/female is about 63/67 years, and the probability of dying under five years in 2012 was 160/1000 live births. These and other factors contribute to Yemen's low ranking in the Development Index cited in the World Human Development Report - 160 among the 162 countries that were rated in the year 2013[20].

Yemen introduced universal immunization against HBV for infants and high risk groups in early 2000, but feed-back on the coverage rate of vaccination and its efficacy in the community have been ignored for a long period. In addition, there has been inadequate information on the prevalence and risk determinants of viral hepatitis as well as on vaccination coverage rate among children in Yemen. The vaccines are provided by the UNICEF from different reliable sources.

b) Study populations and Sample size

This cross sectional study was carried out during 3 months, starting in January 2016 and ending in March 2016, after the approval of the Department of Medical Microbiology, in the Faculty of Medicine and Health Sciences, Sana'a University to the study proposal. A consent form was filled by the parents for each participant. The sample sizes for or study was calculated as follow: First we considered the rate of HBV in Sana'a city, difference (worst acceptable result higher or lower the true rate) and confidence interval as 2%, 0.5% and 95% respectively. According to our calculations, a sample size of at least 752 subjects was required from the population of children under 11 years in Sana'a city (639358 children) [20] which the sample will be selected; this was selected by systematic random method. All health centers and primary schools in Sana'a were listed (27 centers, 33 schools), then by simple random selection 4 of these centers and 4 of these schools were selected; finally, every 5th child admitted to these health centers for normal check and vaccination was selected (about 17% of male children and 13% of female children who refused to donate blood were excluded), and, every 5th child in the selected classes was selected (about 7% of male children and 6% of female children who refused to donate blood were excluded). Second, we considered the rate of HBV in Shabwah governorate, difference (worst acceptable result higher or lower the true rate) and confidence interval as 3%, 0.5% and 95% respectively. According to our calculations, a sample size of at least 178 subjects was required from the population of children under 16 years in Shabwah governorate (255600 children) [20] which the sample will be selected; this was selected by systematic random method. All health centers and primary schools in Atag, Bayhan and Mayfa'a in Shabwah governorate were selected (3 centers, 4 schools), then every 5th child admitted to these health centers for normal check and vaccination was selected (about 36% of male children and 39% of female children who refused to donate blood were excluded), and, every 5th child in the selected classes was selected (about 14% of male children and 9% of female children who refused to donate blood were excluded).

c) Data collection

A full history was taken from each studied individual or from parents; and the findings were recorded in a predesigned questionnaire. The data collected included name, age at the time of the study, sex, residence, status and risk factors of HBV contracting; and laboratory results.

d) Laboratory tests

Capillary blood or vein puncture of whole blood was collected; then sera were separated and tested for HB surface antigen by an Enzyme-linked Immunosorbant assay (ELISA) using a commercially available kit provided by Biokit, Spain. Specimens which proved repeatedly reactive by EIA in two separate tests were considered positive for hepatitis B surface antigen. In addition HB genome was tested for positive HB surface antigen specimens to confirm positivity using a commercial polymerase chain reaction (PCR) -based test (Taqman amplicor, Roche, USA) and all were positive.

e) Statistical analysis

Personal and clinical data were obtained from each subject and recorded into a pre- designed questionnaire, then the data were statistically analyzed by a software version for statistical significance (Epi Info version 6, CDC, Atlanta, USA). From two-by-two tables, the odds ratios were calculated and *P*-value was determined using the uncorrected chi square test. Fisher's exact test was used for the small expected cell sizes with a two-tailed probability value.

III. Results

Tables 1 and 2 outline prevalence and the odds ratio (OR) estimates by their 95% confidence intervals (95% CI), and by Fisher's exact test for cell value less than 5, for positive serological tests of hepatitis B virus and expected risk factors of contracting Hepatitis B virus, and with statistically significant *P*-value using uncorrected chi-square test. The crude seroprevalence

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among children in Sana'a city was 1.8% and it was 3.8% for children from Shabowah governorate. When the age of children was considered, the highest rate of HBV among children in Sana'a city was in age group 9-10 years (2.3%), with associated OR equal to 2.7, CI=0.3 - 25, but this result was not statistic significance (p=0.3). The lowest rate of HBV among children in Sana'a city was in age group 1-2 years (0.85%) (Table 1).

The highest rate of HBV among children in Shabwah governorate was in age group 11-15 years (5.1%), with associated OR equal to 2.4, CI=0.29-21, but this result was not statistic significance (p=0.39). The rates of HBV in Shabwah in age groups 1-5 years and 6-10 years were similar (2.1%). In conclusion there was non-significant effect of older age on contracting hepatitis B virus in both selected area children (Table 1, 2).

In the case of risk factors of hepatitis B virus infection for children in Sana'a city, there was a significant association of non vaccination to HBV vaccine (OR=4.2, CI=1.23-15.9, p=0.007), and with history of parental exposure (OR=4.05, CI=1.1-14.3, p=0.01). Also there was not significant association of birth by cesarean in which OR=3.3, CI=0.0-16, p=0.1) and birth in hospital (OR=1.27, CI=0.41-3.9, p=0.64) (Table 1). In the case of risk factors of hepatitis B virus infection for children in Shabwah governorate, it was found that there was a highly significant association of contracting HBV infection with non vaccination to HBV vaccine with significant rate equal to 5.5% (OR and CI=undefined, p=0.045), birth in hospital (OR=5.8, CI=1.01- 31.4, p=0.01) and birth by cesarean (OR=5.6, CI=0.7-38.7, p=0.02), but not significant with history of parental exposure (OR=2.24, CI=0.5 -11, p=0.25) (Table 2).

Table 1: Prevalence of HB surface antigen in different age groups and risk factor analysis of HBV in a sample of children under 10 years old, in Sana'a city, Yemen Factors

Age groups	HB S Ag positive		*OR*	*CI*	*P value*
	No.	%			
1-2 years (n=119) Reference	1	0.85			
3-5 years (n=274)	5	1.8	2.1	0.25-18.9	0.46
6-8 years (n=273)	5	1.8	2.2	0.25-19	0.46
9-10 years (n=174)	4	2.3	2.7	0.3- 25	0.3
Total (n=840)	15	1.8			
Factors					
Vaccinated to HBV Yes (n=504) Reference	4	0.79			
No (n=336)	11	3.3	4.2	1.23-15.9	0.007
Birth in hospital (n=334)	7	2	1.27	0.41-3.9	0.64
No (n= 506) Reference	8	1.6			
Birth by cesarean Yes (n=39)	2	5.1	3.3	0-16	0.1
No (n= 801) Reference	13	1.6			
Parental exposure Yes (n=72)	4	5.6	4.05	1.1-14.3	0.01
No (n= 768) Reference	11	1.4			

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Table 2: Prevalence of HB surface antigen in different age groups and risk factor analysis of HBV in a sample of children under 15 years old, in Shabowah governorate, Yemen Factors

Age groups	HB S Ag positive		*OR*	*CI*	*P value*
	No.	%			
1-5 years (n=47) Reference	1	2.1			
6-10 years (n=48)	1	2.1	0.97	0.05-16.1	0.98
11-15 years (n=117)	6	5.1	2.4	0.29- 21	0.39
Total (n=212)	8	3.8			
Factors					
Vaccinated to HBV Yes (n=69) Reference	0	0			
No (n=143)	8	5.5	Undefined		0.045
Birth in hospital Yes (n=22)	3	13.6	5.8	1.01-31.4	0.01
No (n=190) Reference	5	2.6			
Birth by cesarean Yes (n=13)	2	15.4	5.6	0.7-38.7	0.02
No (n= 199) Reference	6	3			
Parental exposure Yes (n=67)	4	6	2.24	0.5-11	0.25
No (n= 145) Reference	4	2.8			

IV. Discussion

The prevalence rate of HB surface antigen in our study was variants among selected healthy children in the two selected areas these differences in the prevalence rates might be the geographical differences and the national immunization programmes vaccination coverage in the capital city of Sana'a (high) and urban area of Shabowah (low) and or related to the differences in the classification of age groups. The prevalence rate in our study in Sana'a city was 1.8%, is lower than that reported among infants in Sana'a city previously where the rate was 4.1% but the rate in Shabwah (3.8%) is roughly similar to that reported previously in Sana'a city among infants [10]. Although it is difficult to compare the prevalence rates reported in our study (among children), with that reported by Al-Shamahy *et al*. [10, 11] (among children and mothers and among blood donors etc), it seems that the rate of HBsAg has decreased dramatically. Introducing hepatitis B vaccine within the national immunization programmes improvement of the people's knowledge about hepatitis risk factors through educational programmes, and the availability of measures to diagnose hepatitis in health centers and blood banks might explain this decrease [16,18, 21].

The rates of HBV in our study was higher than that reported in Northern, Western, and central Europe, North America, and Australia, children and general population where the rates of HBV surface antigen was ranged from 0.2-0.5%, [22]. In other hand the crude rate of HBV surface antigen in our study was similar to that reported in Eastern Europe, the Mediterranean, Russia and the Russian Federation, Southwest Asia, Central and South America among children general population where the rates of HBV surface antigen was ranged from 2 -7 %, [23], but lower than that reported in Parts of China, Southeast Asia, and tropical Africa among general population where the rates of HBV surface antigen was ranged from 8-20%[22]. These differences in the prevalence rates might be explained by the geographical differences in the availability of services and vaccination programmers.

Many other studies in nearby countries have shown a lower prevalence of hepatitis B among children, as Saudi Arabia (0.05%) [24], This may be because the good availability of services and vaccination programmers in Saudi Arabia and there is insufficient protection for patient children admitted to hospitals in Yemen, since sterilization, disinfection and general standards of training and proficiency are generally lacking in most hospitals in Yemen.

HBV infection effects all ages everywhere[22-26]. There was slightly trend toward increased levels of HB surface antigen with the older children where prevalence rate is ranged from 0.84% in 1-2 years group to 2.3% in 6-8 years group in Sana'a city and this trend toward increased levels of HB surface antigen with the older children is more clear in Shabowah governorate where the rate is ranged from 2.1% in 1-5 years old to 5.1% in 11-15 years group (tables 1, 2). The increasing of prevalence rate with increasing age in our study could indicate an accumulation risk of infection over time. In addition, the results indicated that horizontal spread of HBV may be of greater importance than vertical transmission.

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The study illustrates that children in Yemen mainly in rural areas as Shabowah governorate are at a high risk of becoming infected in their early years. The first risk for infection occurs in the first few days spent in hospitals during normal delivery (OR=5.84, p=0.01) or by cesarean section (OR=5.6, p=0.02), and this confirms that use of unsterilized or inadequately sterilized contaminated instruments are a possible route of infection. It is possible that there was insufficient protection for children admitted to hospitals in Yemen. Sterilization, disinfection and general standards of training and proficiency are generally deficient in most hospitals in Yemen particularly in rural areas.

The rate of HBV infection was higher in Shabowah area (3.8%) than in Sana'a city this regional variation might be due to non-uniformities in immunization and engagement in risky behaviors across different sites. Also our study shows the important of HBV vaccine in prevent infections (table 1,2) in which higher risk of contracting HBV infection among non-vaccinated children and more HBs Ag-positive cases were from unvaccinated children and rural area suggesting of poorer vaccination coverage of the rural population.

Evidence from these studies in Yemen suggests that there is a steady increase in exposure to HBV over a lifetime. Hospital-acquired HBV infection is very common in Yemen, and prevention is eventually possible by applying standard policies of sterilization, disinfection and personal training to implement this policy and guarantee refinements in the screening of blood donors. In Yemen, vaccination should be considered for all children and programs to immunize all newborn Babies with a goal of 80% coverage or more should be performed in the next 2 to 4 years particularly in rural areas, in the same with health education.

V. Conclusion

There was a significant association of non-vaccinated children, birth by cesarean, and with history of parental exposure with contracting HBV infection. Evidence from these studies in Yemen suggests that there is a steady increase in exposure to HBV over a lifetime. Hospital-acquired HBV infection is very common in Yemen, and high vaccination coverage rate should be achieved particularly in rural areas, in parallel with health education.

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Conflict of Interest

"No conflict of interest associated with this work".

Author's Contribution

This research work is part of 2 M.Sc. thesis. The candidates are the fourth and fifth authors (BBMA) who conducted the field works and the experiments and wrote up the thesis. The corresponding author (HAA) supervised the experimental work, revised and edited the thesis draft and the manuscript. (BMJ) and (AGA) were co-advisor of the works, and helped in supervised the experimental work, revised and edited the thesis draft and the manuscript.

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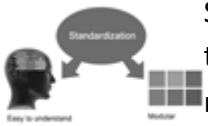


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Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).



The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.





The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.



The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.



After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:



Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.



Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

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Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



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- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

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

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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

General style:

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

To make a paper clear: Adhere to recommended page limits.



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Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

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

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

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

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

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

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



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Results:

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

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

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- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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