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Human Immunodeficiency Virus Infectious Profile Change in Mali: A Narrative Review

By Nouhoum Bouare, Sebastien Bontems & Christiane Gerard

Abstract- West Africa is reputed as an epicenter of HIV-2 infection. Studies undertaken in Mali suspected HIV-1 more prevalent. Our study aims to document HIV infectious profiles in Mali and analyze HIV-1 dominance. We documented HIV studies undertaken in Mali from 1985 to 2010. We proceeded to a bibliographic search focused on theses from the Medicine Pharmacy Odontostomatology Faculty (FMPOS) of Bamako, survey reports, and abstracts or papers published in reviews with the reading committee. Documents were physically and virtually (via website) consulted and exploited. We gave preference to studies that discriminated against HIV serotypes. The data were analyzed according to study population/publication, representativeness, infectious profiles reporting, socio-demographic and clinical characteristics. HIV profiles variation in space and time was analyzed by using a linear regression model. Calculations were done using Excel software.

Keywords: *epidemiology, HIV infection, serotypes change, Mali, West Africa.*

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Nouhoum Bouare ^α, Sebastien Bontems ^σ & Christiane Gerard ^ρ

Abstract- West Africa is reputed as an epicenter of HIV-2 infection. Studies undertaken in Mali suspected HIV-1 more prevalent. Our study aims to document HIV infectious profiles in Mali and analyze HIV-1 dominance. We documented HIV studies undertaken in Mali from 1985 to 2010. We proceeded to a bibliographic search focused on theses from the Medicine Pharmacy Odontostomatology Faculty (FMPOS) of Bamako, survey reports, and abstracts or papers published in reviews with the reading committee. Documents were physically and virtually (via website) consulted and exploited. We gave preference to studies that discriminated against HIV serotypes. The data were analyzed according to study population/publication, representativeness, infectious profiles reporting, socio-demographic and clinical characteristics. HIV profiles variation in space and time was analyzed by using a linear regression model. Calculations were done using Excel software. Out of 17 studies that reported HIV profiles, nine documented in full serotypes profiles. They mainly concerned health care patients and prostitutes, as they are likely more exposed to HIV infection. The sexual route was mostly described. In prostitutes group, significant regression of HIV-2 was observed between 1987-1989 and 1995 (65/517 vs 7/176) ($p = 0.001$) while HIV-1 increased (36/517 vs. 63/176) ($p < 0.0001$). The chronology of events showed prior existence of both profiles but with an initial dominance of HIV-2. The study surprisingly highlighted HIV-1 profile dominance in Mali, whereas West Africa is reputed as an HIV-2 epicenter. However, it suffered lack of representativeness of preliminary studies. HIV profile change and propagation seem essential due to the sexual route in this country.

Keywords: epidemiology, HIV infection, serotypes change, Mali, West Africa.

I. INTRODUCTION

West Africa is reputed to be the epicenter of HIV-2 infection [1]. This HIV profile was also endemic in the same geographic area [2]. In Mali, the first AIDS case was identified in 1985 [3]. In this country, the early studies reported a dominance of HIV-2 on HIV-1 [4, 5, 6, 7]. However, an anterior study conducted in patients admitted in *pneumophthisiology* setting revealed HIV-1 more prevalent in the sub-study population of non-tuberculosis patients [8]. Unlike the

prior studies, more or less recent works conducted in this country reported an opposite trend [9-20]. However, in the country, a significant higher HIV-2 prevalence was observed in 2010 in older women than in young ones (in 2009), despite a high HIV-1 dominance in the both populations [19]. This HIV-2 trend in older adults contrasting with the low trend in young ones, aroused our curiosity to analyze the dominance of the HIV-1 infectious profile that seems plausible in Mali.

II. METHODOLOGY

a) Procedure

This narrative review consisted of analyzing the data from preview studies concerning HIV infection in Mali. We have pursued a bibliographic search focused on HIV studies (subject or not to publication in scientific reviews) undertaken in Mali from 1985 to 2010. The FMPOS theses file, as well as papers related to HIV/AIDS topic, were consulted and exploited for data collection and analysis. We prioritized studies having documented the serotypes profiles (HIV-1, HIV-2, and HIV-1/2), by using a discriminatory or confirmatory test. We structured the argumentation around the following criteria: study period, publication date and reference; study population including hospital patients, prostitutes, pregnant women, blood donors, general population; study sample size; study population characteristics such as ages, average age, gender, underlying diseases, clinical symptoms, risk factors; stratification by age (<50-years-old and >50-years-old); testing for HIV serotypes profiles discrimination using immunochromatography, Western Blot or Line Immunoassay principle; typology of the publication such as abstracts or full text from international journals, meetings or conferences presentations, theses and reports.

b) Statistical Analysis

Results are presented as mean \pm SD (range) for continuous variables and frequencies (%) for categorical variables. Categorical variables were compared between the groups using a chi-square test. Results were significant at the 5% level ($p < 0.05$). Linear Regression model was used to analyze the HIV profiles trends. Calculations were done using Excel Software.

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c) *Human Subjects*

This proposed study uses an anonymous secondary data set, and does not qualify as human subject research.

III. RESULTS

A total of 17 studies were exploited. They mainly concerned health care patients and prostitute women populations (Table 1), as they are likely to be more exposed to HIV infection than the general population. Samples size in these studies ranged from 23 to 3179 subjects (Table 2). Regarding the stratification of population by age (<50-years-old versus >50-years-old), a study revealed that despite HIV-1 prevalence was high in both strata, HIV-2 was significantly more prevalent in the older populations than in younger (2/1000 vs. 5/231) ($p = 0.0003$). Out of 17 studies reviewed nine only documented in full HIV serotype profiles from the abstract and/or full text (Table 2). This table also informs on HIV prevalence that ranges between 0.73% and 75.79%. The lowest prevalences were observed in blood donors and pregnant women. The prostitutes and health care patients were the most affected. When one considers only the prostitutes populations (Table 2), a significant regression of HIV-2 can be observed between 1987-1989 and 1995 (65/517 vs. 7/176) ($p = 0.001$). Conversely, HIV-1 increased significantly during the same period (36/517 vs. 63/176) ($p < 0.0001$). As far as health care patients are only concerned, there was a significant increase in HIV-1 ($Y_{\text{HIV-1}} = 9.20x + 22.80$; $R^2 = 0.6351$) while HIV-2 significantly regressed ($Y_{\text{HIV-2}} = -3.81x + 34.47$; $R^2 = 0.2895$). Furthermore, when taking into account the overall population, a similar trend can be observed ($Y_{\text{HIV-1}} = 8.48x + 16.38$; $R^2 = 0.646$) vs ($Y_{\text{HIV-2}} = -5.626x + 55.82$; $R^2 = 0.3321$). The chronology of events, as well in all the populations studied as in health care patients taken alone (Table 2 and 3; fig1 and 2), shows that both infectious profiles have pre-existed in Mali, but with an initial predominance of HIV-2 and change toward HIV-1 that occurred probably between 1990 and 1994.

IV. DISCUSSION

A Malian study reported a higher HIV seroprevalence in prostitutes in 1991 (70%) [21]. In Mali, HIV prevalence of 4.1% (41/1000) was measured in 2009 in pregnant women (young women), with a higher dominance of HIV-1 (95%) [19, 22]. This seroprevalence measured in 2009 in the Bamako district was comparable to 3.5% (183/5224) reported in 2006 in pregnant women recruited from seven locations (including Bamako) across the country [23]. Likewise, in 2010, HIV seroprevalence 6.1% (14/231) measured in older women did not differ from 4.1% reported in young ones [19, 22]. By contrast, the proportion of HIV-2 was significantly higher in older women than in younger

ones, 2.16% (5/231) vs. 0.2% (2/1000); $p < 0.001$. The HIV epidemiological profile between 1985 and 2010 shows at the beginning of this observation period HIV-2 dominance; a trend that has been reversed later in favor of HIV-1, which is still dominant today. Indeed, several studies have revealed the dominance of HIV-1 between 1988 and 2010 [9-20], unlike the first studies undertaken in Mali between 1985 and 1989 [4, 5, 6, 7]. This new trend in favor of HIV-1 dominance contrasts *a priori* with evidence that West Africa is the epicenter of the epidemiology of HIV-2 [1]. Our work is limited by the lack of representativeness from some preliminary studies undertaken and reported in Mali. It suffered equally from the data insufficiency related to HIV infectious profiles in some documents consulted. Guinea-Bissau (a West African country) is described as the epicenter of the HIV-2 epidemic [24]. In the same country, HIV-1, HIV-2 and HIV-1/2 seroprevalence were respectively 1.1%, 8.4% and 0.1% for the period of 1992-1995 and 7.7%, 5.1% and 1.9% in 2005 [25]. Between February 1987 and May 1988, the Central Hospital of Dakar registered HIV-1 frequency comparable to that of HIV-2 46% (50/109) vs 40% (44/109); $p > 0.05$ [26]. In the same city, prevalence rates for HIV-1 (6%), HIV-2 (3.6%) and HIV-1/2 (0.4%) were reported, in 2000, among sex workers [27]. In Ivory Coast, a predominance of HIV-1 was reported in 1988 [28]. In Mali, a prior study carried out in patients enrolled in a specialized hospital reported in none tuberculosis patients a rate of 5.5% (9/164) for HIV-1 vs. 1.22% (2/164) and 1.83% (3/164) respectively for HIV-2 and HIV-1/2 [8]. However, considering the totality of patients with or without tuberculosis, the frequencies were 4.58% (22/480), 2.71% (13/480), and 3.96% (19/480), respectively for HIV-1, HIV-2, and HIV-1/2. In this country, a high frequency of HIV-1 was reported in 2009 among students [20]. Bouare et al. demonstrated that HIV-2 was significantly more common in older women than in younger ones [19]. Suggesting HIV-2 infection occurred earlier (probably 20 years or more) in these older adults infected. That may explain and confirm two hypotheses: HIV-2 infection oldness and HIV infectious profile change toward HIV-1 in Mali. Moreover, from 1988 to 1992, we observe a quantitative dominance of HIV-1 2.99% (71/2378) vs. 0.97% (23/2378) and 1.39% (33/2378) respectively for HIV-2 and HIV-1/2 [9]. A study conducted between 1990 and 1999 even reported a predominance of HIV-1 with a prevalence of 58.55% (462/789) vs. 5.58% (44/789) and 11.66% (92/789) respectively for HIV-2 and HIV -1/2 [10]. It also described the growing trend of emigration between 1993 and 1998 (4.18% to 8.11%), a sexual transmission rate of 98.10%, the first peak of HIV-1 in 1992, and persistent latency observed for HIV-2. This rate of 98.10% of sexual transmission is supported by Bouare et al. [22], who reported that HIV transmission might be essentially sexual in Mali. The data for the study

between 1987 and 1989 [6, 7] attributed a significant proportion of HIV infection linked to staying (since 1980) in Central Africa, West Africa, and Europe. This could partially explain the foreign exposition and contamination of the people before they come back in Mali. Other studies in Mali focused on prostitution which can explain the spread of HIV infection [4, 5, 6, 13, 14, 21]. One of them reported that the highest prevalence was 70% among registered prostitutes in 1991, and most regions of Mali had experienced higher HIV prevalence among sex workers in 1992 compared to 1988 [21]. Also, a bibliographical study of the period 1983 to 2003 reported in 2004 the dominance of HIV-1 since 1990 and HIV-2 dominance before that time [14]. It also pointed out limitations such as poor access to studies, especially that of NGOs (Non-Governmental Organizations), and insufficient data regarding some summaries in general. Through a study conducted in 1995 in Mali regarding prostitutes mainly composed of foreign (including Nigerian and Ghanaian), Peeters and coworkers reported a significant increase in HIV-1 against a decrease of HIV-2 [13]. They also reported the similarity of this trend with those observed in the neighboring countries of Mali. They hypothesized recent contamination among women who started sex work a year (or less than a year) before they conducted their study since HIV-1 subtype G was detected. As for our study, when we consider only the population of prostitute women, significant regression of HIV -2 is observed between the periods 1987 to 1989 and 1995 [12.57 % (65/517) vs. 3.98% (7/176)]; $p = 0.001$. Conversely, HIV-1 increased significantly during the same period [6.96% (36/517) vs. 35.79% (63/176)]; $p < 0.0001$. This is further corroborated and confirmed by the linear regression analysis related HIV infectious profile change in the both patient population ($Y_{HIV-1} = 9.20x + 22.80$, $R^2 = 0.6351$; $Y_{HIV-2} = -3.83x + 34.47$, $R^2 = 0.2895$) and all the combined populations ($Y_{HIV-1} = 8.48x + 16.38$, $R^2 = 0.6459$; $Y_{HIV-2} = -5.626x + 55.82$, $R^2 = 0.3321$). From the above, we suggest that the reversal of the epidemiological profile of HIV for HIV-1 probably occurred in Mali between 1990 and 1994, while Antonio Biague et al. described the HIV-1 increase and HIV-2 decline between 1992-1995 and 2005 [25]. In HIV epidemiological study context, documenting of all serotypes profiles (HIV-1, HIV-2, and HIV-1/2) and genotypes in both abstract statement and full text (usually difficult to access) are needed to track their evolution in space and time and enable more precise dating of infectious profiles to change.

In conclusion, this present work surprisingly highlighted HIV-1 profile predominance in Mali, whereas West Africa is reputed to be the HIV-2 epicenter. The HIV profile change seems to occur between 1990 and 1994. The transmission risks and routes such as sexual, trip duration and emigration are *a fortiori* highlighted. The

propagation of HIV infection seems essentially linked to the sexual route in this country.

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Table 1: Chronology of events according to the publication date and study population characteristics

Publication	Study Population Characteristics		
Date	Population	Age (mean±SD)	Risk Factors and other informations
1987	Prostitutes, Prisoners, Patients, Pregnant women (PW)	26	Prostitution, homosexuality, transfusion
1988	Prostitutes	35	Prostitution
1988	Patients	35	Voyage (stay at foreign)
1989/1993	Prostitutes, Patients, Prisoners, Women, Men	30.18	Prostitution (stay at foreign), widowhood, divorce, residence, tattoo, not condom use
1993	Patients		Peasants, Traders, Big travelers
1998	Prostitutes	28.8	Prostitution
2000	Patients (AIDS)		
2001	Patients	35.19±9.45	Sex transmission, emigration; first peak HIV-1 (1992) and HIV-2 latency
2001	Blood donors (BD)		Absence of discriminant test in 93 and 99, HIV-1 predominant (94-98)
2001	Patients, Prostitutes, PW, BD		Prostitution (HIV seroprevalence: 70%)
2004	Bibliographic studies of theses		Groups at risk: prostitutes, ambulatory saleswomen, coaxers, truck drivers; lack studies access, data lack in some abstracts
2004	Patients	37.5 ± 7.93	Stay at foreign
2006	General population		
2006	Patients (children)	7	
2009	Students		More HIV-1 than HIV-2
2012/2013	Pregnant women / Patients	25.2±6.3/62.1±8.6	Not condom use, divorce, voyage
2013	Patients	35.2±9.4	Patients (Predominantly rural, female and young); Stage III WHO (64.5%)

WHO: World Health Organization

Table 2: Prevalence of HIV infection according to the study period and population

			Sample size	Serotypes HIV (%)*			HIV Frequences	P
Date	Period	Population	N	HIV-1	HIV-2	HIV-1/2	n (n1; n2; n1/2)	(%)
1987	1	Prostitutes	30	10,53	78,95	10,53	19 (2 ; 15 ; 2)	63,33
1987	1	Prisoners	23	33,33	33,33	33,33	3 (1 ; 1 ; 1)	13,04
1987	1	Patients	42	33,33	66,67	0	9 (3 ; 6 ; 0)	21,43
1987-1988	2	Patients	480	40,74	24,07	35,19	54 (22 ; 13 ; 19)	11,25
1987-1988	2	Patients	316	32,5	27,5	40	40 (13 ; 11 ; 16)	12,66
1987-1988	2	Patients	164	64,29	14,29	21,43	14 (9 ; 2 ; 3)	8,54
1987-1989	3	Prostitutes	487	27,64	40,65	31,71	123 (34 ; 50 ; 39)	25,26
1987-1989	3	Prisoners	496	33,33	55,56	11,11	18 (6 ; 10 ; 2)	3,63
1987-1989	3	Patients	866	31,4	46,28	22,31	121 (38 ; 56 ; 27)	13,97
1987-1989	3	Pregnant women	588	22,22	77,78	0	9 (2 ; 7 ; 0)	1,53
1987-1989	3	Blood donors	687	60	20	20	5 (3 ; 1 ; 1)	0,73
1987-1989	3	Travellers	372	47,37	42,11	10,53	19 (9 ; 8 ; 2)	5,11
1987-1989	3	Women	1578	25,81	48,92	25,27	186 (48 ; 91 ; 47)	11,79
1987-1989	3	Men	1903	40,37	37,61	22,02	109 (44 ; 41 ; 24)	5,73
1987-1989	3	Housewives	780	18,75	64,58	16,67	48 (9 ; 31 ; 8)	6,15

1987-1989	3	People <50 years old	3179	31,05	44,77	24,19	277 (86 ; 124 ; 67)	8,71
1987-1989	3	People >50 years old	264	41,67	41,67	16,67	12 (5 ; 5 ; 2)	4,55
1988-1992	4	Patients	2378	55,91	18,11	25,98	127 (71 ; 23 ; 33)	5,34
1988-1992	4	Patients	127	55,91	18,11	25,98	127 (71 ; 23 ; 33)	N/A
1990-1999	5	Patients	789	77,26	7,36	15,38	598 (462 ; 44 ; 92)	75,79
1995	6	Prostitutes	176	77,78	8,64	13,58	81 (63 ; 7 ; 11)	46,02
2003	7	Patients	71	87,32	8,45	4,23	71 (62 ; 6 ; 3)	N/A
2004-2005**	8	Patients	81	98,77	1,23	0	81 (80 ; 1 ; 0)	N/A
2009-2010**	9	Pregnant women	1000	95,12	4,88	0	41 (39 ; 2 ; 0)	4,1
2009-2010**	9	Patient women>5 0 years old	231	64,29	35,71	0	14 (9 ; 5 ; 0)	6,06

* Data columns for figure1

**Study without HIV1/2 data (not include in figure1 data)

N/A: not applied (because study population includes HIV patients only)

n (n1; n2; n1/2): frequencies of HIV (HIV-1; HIV-2; HIV-1/2)

P: prevalence

%.: percentage

Table 3: Proportion of HIV-1, HIV-2 and HIV-1/2 infections in health care patients according to the study period

Date	Period	HIV-1 (%)	HIV-2 (%)	HIV-1/2 (%)
1987-1988	2	40,74	24,07	35,19
1987-1988	2	32,50	27,50	40,00
1987-1988	2	64,29	14,29	21,43
1987-1989	3	31,40	46,28	22,31
1988-1992	4	55,91	18,11	25,98
1990-1999	5	77,26	7,36	15,38
2003	7	87,32	8,45	4,23

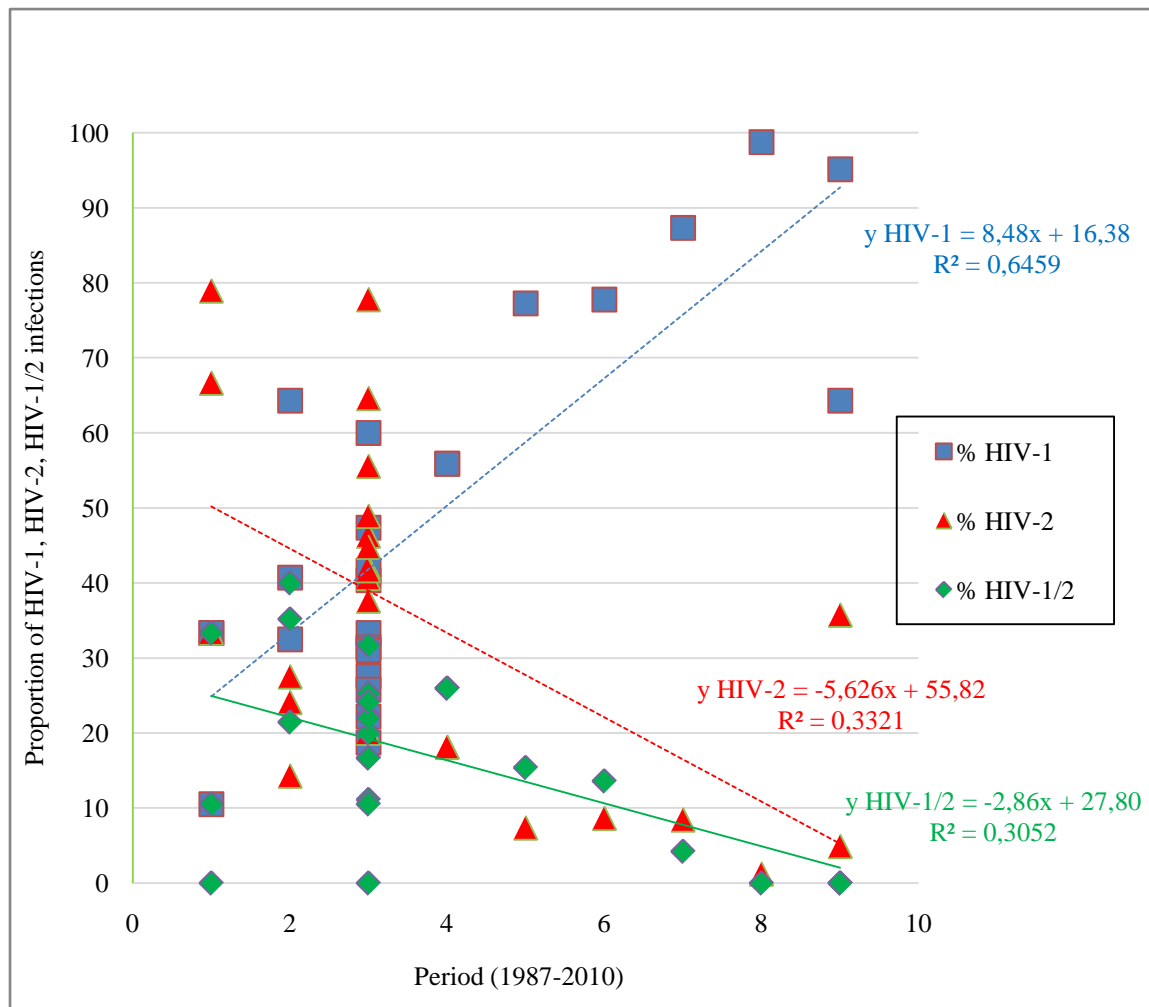


Figure 1: The trends of HIV infectious profiles in full populations studied (field Mali) in space and time

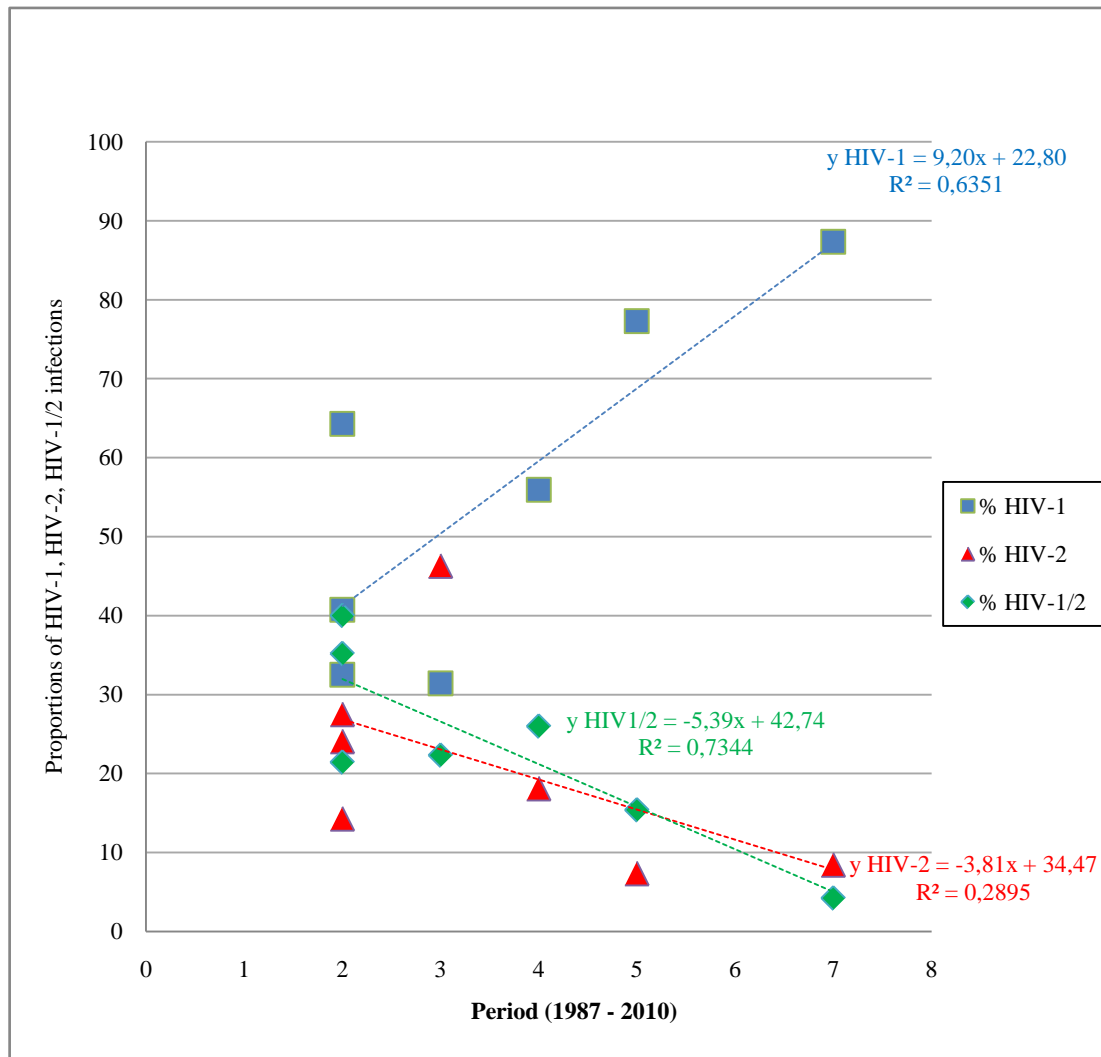


Figure 2: The trends of HIV infectious profiles in sick patients (field Mali) in space and time



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Characterization and Antibiotic Sensitivity Testing of Clinical Bacteria Species Isolated from Kunu Drink Sold in Rumuolumeni, Rivers State

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Abstract- The isolation, characterization and antibiotic sensitivity tests of some clinical bacteria species isolated from Kunu drink sold in Rumuolumeni, Rivers State was carried out. Samples of the Kunu drink was bought from vendors indifferent locations and their bacteriological counts enumerated using standard microbiological methods by the pour plate technique. The antibiotic sensitivity pattern of the pure bacteria isolates against some antibiotics was determined using the disc diffusion method. The total bacterial counts of the Kunu in the different locations ranged from 4.0×10^2 to 8.6×10^2 cfu/ml. Four species of bacteria including *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Streptococcus* spp were isolated and identified by grain staining and their biochemical reactions. The most prevalent isolate in terms of occurrence was *Escherichia coli* (50%) followed by *Enterobacter aerogenes* (30%), *Staphylococcus aureus* (10%) and *Streptococctts* spp (10%).

GJMR-C Classification: NLMC Code: QW 50



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Abstract- The isolation, characterization and antibiotic sensitivity tests of some clinical bacteria species isolated from Kunu drink sold in Rumuolumeni, Rivers State was carried out. Samples of the Kunu drink was bought from vendors indifferent locations and their bacteriological counts enumerated using standard microbiological methods by the pour plate technique. The antibiotic sensitivity pattern of the pure bacteria isolates against some antibiotics was determined using the disc diffusion method. The total bacterial counts of the Kunu in the different locations ranged from 4.0×10^2 to 8.6×10^2 cfu/ml. Four species of bacteria including *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Streptococcus* spp were isolated and identified by grain staining and their biochemical reactions. The most prevalent isolate in terms of occurrence was *Escherichia coli* (50%) followed by *Enterobacter aerogenes* (30%), *Staphylococcus aureus* (10%) and *Streptococcus* spp (10%). The antibiotic tests showed that *Escherichia coli* had high resistance to Chloramphenicol (70%), followed by Septrin (62.7%) and Sparloxacin (62.7%), while *Enterobacter aerogenes*, *Streptococcus* spp and *Staphylococcus aureus* had low rates of resistance to all the antibiotics tested. The results of this study demonstrated that Kunu drink sold in Rumuolumeni was contaminated with potentially pathogenic bacteria species, including antibiotic resistant *E. coli* and these may lead to failures in antibiotic chemotherapy among consumers of the product if appropriate safety and regulatory measures are not adopted.

I. INTRODUCTION

According to Maji *et al.*, (2011), Kunu drink is a locally prepared indigenous non-alcoholic beverage normally prepared and consumed in large quantity in Nigeria, especially in the northern part of the country (Amusa and Aswaye, 2009). It can be consumed during the wet and dry seasons due to its thirst quenching properties. Umaru *et al.*, (2014) reported that Kunu drink is sold in many public places such as markets, offices, schools, motor parks and as drinks during festival, weddings and naming ceremonies. It is an appetizer and food complement used to quench hunger (Adelekan *et al.*, 2014). Kunu drinks are usually

produced using maize, guinea corn, millet or sorghum in varying proportions (Maji *et al.*, 2011) to which sweet potato sometimes is added to increase the taste of the Kunu, which is a major factor that attracts consumers to the product. This common drink is usually packaged and sold in 50ml to 1L plastic bottle and at times tied in some disposal polythene bags the drink is mostly consumed within 20-35 hours of production due to its poor keeping quality (Akoma *et al.*, 2012). This drink is not expensive because the grains and other ingredients used for production are locally sourced. The packaging materials are also readily available, cheap and affordable within the communities.

Different workers have reported that Kunu is rich in vitamins, minerals, carbohydrates and proteins (Adebayo *et al.*, 2010; Essien *et al.*, 2009; Folasade & Oyenike, 2012). Oluwajoba *et al.*, (2013) also noted that the nutritional content of Kunu drink include protein (2.31-3.63%), fat (3.55-3.63%), ash (1.66-1.21%) and carbohydrate (82.92-83.55%). The health benefits of kunu drink is that, it lowers blood pressure and promotes good functioning of the heart, improves healthy pregnancy and adequate breast milk flow, boosts sperm count in men, relaxes personal mood and promotes good sleep and reduces menstruation pains for women.

The local kunu drink could act as a vehicle for food borne infections like Brucellosis, Tuberculosis, Shigellosis, Listeriosis and *Staphylococcus* etc. Most of the pathogens found in the drink such as *Staphylococcus* sp, *Bruce1la* sp, *Pseudomonas* sp, *Clostridium*, *Salmonella*, *Vibrio cholerae* and *Escherichia coli* can lead to change in the physical and nutritional qualities of kunu. Also, activities of the natural food enzymes could also contribute in the spoilage of the final product. The high water content (about 85%) coupled with crude method of production and packaging under inadequate sanitary conditions can also predispose kunu drink to microbial contamination (Aya *et al.*, 2010).

According to Mbachu *et al.*, (2014), the short life of kunu drink is a major problem faced by the producers and consumers. The introduction of microbes into kunu

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drink results from the processing activities and materials used such as water, handling and presentation techniques. The water content coupled with the crude method of production and packaging under poor sanitary conditions predisposes the drink to sudden microbial contamination (Akoma *et al.*, 2012). Again, there is no public health regulatory agency that monitors the production processes in spite of the associated harm that kunu drink causes Bukar *et al.*, (2010). The aim of this study is to characterize and determine the antibiotic sensitivity of some pathogenic bacteria species isolated from Kunu drink sold in Rumuolumeni, Rivers State.

II. MATERIALS AND METHODS

a) Study Area

The study was conducted in Rumuolumeni, Port Harcourt, Rivers state, Nigeria. Port Harcourt lies between latitude 4°46'38.71"N and longitude 7°00'48.24"E. and located in the tropical rainforest in Nigeria.

b) Collection of Sample

Five bottles of hawked kunu samples were bought from vendors in different locations in Rumuolumeni, properly labelled, placed in a sterile plastic container and transported to the Biology laboratory, Ignatius Ajuru University of Education for microbiological analysis.

c) Processing of Sample

The samples were mixed gently and 10ml of each was added to 90 ml distilled water with a clean pipette. The solution was mixed and diluted serially by transferring 1 ml of the stock sample into sterilized test tubes containing 9ml of peptone water. The procedure was repeated for the third and fourth test tubes to make a dilution of 10^3 and 10^4 .

d) Preparation of Media

All the glassware used such as petri-dishes, conical flasks, test tubes and pipettes were washed with detergent, rinsed in water, dried and sterilized in the hot air oven at 60°C for 1 hour. Different culture media such as Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar (SSA), Trisulphate Citrate Bile Salt Agar (TCBS)

and Manitol Salt Agar (MSA) were used for isolation. Each of the media was prepared by weighing out appropriate quantities according to the manufacturers instruction and dissolved completely in the required volume of distilled water. The media were autoclaved at 121°C for 15 minutes and allowed to cool at 45-50°C. The media was dispensed aseptically into the petri-dish plates and left on the table to solidify at room temperature.

e) Isolation and Preservation

Using a sterile loop, discrete colonies were all sub-cultured onto another media to obtain pure colonies. This was done by streaking a loopful of a particular isolate into freshly prepared culture media plates for bacteria. The sub-cultured nutrient agar plates were incubated at 37°C. Bacteria pure cultures were accordingly stored in sterile agar slants for preservation and further analysis at 4°C.

f) Identification of Isolates

The isolates were identified using gram staining and biochemical tests such as: motility test, urease test, citrate utilization test, indole test, oxidase test, coagulase test, catalase test, vogues proskauer reaction and methyl red test. Identification was based on comparison of the characteristics of the isolates described by (Chess brough, 2006).

g) Antibiotic Susceptibility Test

The isolates were screened for antimicrobial sensitivity using the Kirby-Bauer agar disk diffusion method (CLSI, 2009). A suspension of each isolate was prepared in peptone water to match 0.5 McFarland turbidity standards in order to standardize the inoculum. The standardized inoculum of each isolate was inoculated onto the surface of plain Mueller-Hinton agar plates and Septrin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (5 µg), Amoxycillin (30 µg), Ciprofloxacin (5 µg) Augmentin (30 µg), Gentamicin (10 µg), Pefloxacin (10 µg), Tarivid (30 µg) and Streptomycin (10 µg) discs were placed and incubated at 37°C for 24 hours. The zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute.

III. RESULTS

Table 1: Total bacteria count of kunu sold at the different location in Rumuolumeni Port Harcourt metropolis

Location	Kunu
A	5.1×10^2
B	6.2×10^2
C	4.6×10^2
D	7.1×10^2
E	4.1×10^2
F	8.5×10^2

Keys: A= Waterside, B = Big tree market, C = Akar Junction, D = Iwofe school gate, E = St. John's, F = Town Hall

The bacterial counts from the different samples of Kunu ranged from 4.6×10^2 cfu/ml (which was the lowest recorded for St. John's) to 8.6×10^2 cfu/ml enumerated in Town Hall.

Table 2: Bacteria isolates identified from Kunu samples in different locations

Bacteria Isolates	Locations					
	A	B	C	D	E	F
<i>Escherichia coli</i>	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	-	-	+	-	+
<i>Streptococcus spp</i>	-	-	-	-	+	+
<i>Enterobacteria aerogenes</i>	+	+	+	-	+	+

Keys: A= Waterside, B = Big tree market, C = Akar Junction, D = Iwofe school gate, E = St. John's, F = Town Hall

Table 2 shows the bacterial species isolated from samples of Kunu drinks sold at the different locations in Rumuolumeni. The isolates were *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus spp.* and *Enterobacter aerogenes*. *Escherichia coli* was the most

predominant isolate with very high percentage occurrence (100%), followed by *Enterobacter aerogenes* (70%) and *Staphylococcus aureus* had the least occurrence (30%).



Plate 1: Bacterial colonies growing on petri dish plates

Table 3: Antibiotic resistance pattern of bacteria isolates from Kunu drink

Percentage Resistance of Isolates				
Antibiotics Conc. (μ g)	<i>Escherichia coli</i>	<i>Enterobacter Aerogenes</i>	Strept. spp	<i>Staphylococcus Aureus</i>
Septtrin30	68.7	14.3	0.0	16.7
Chloramphenicol 30	75.0	21.4	0.0	0.0
Sparfloxacin 5	68.7	35.7	33.3	16.7
Ciprofloxacin 5	50.0	7.1	33.3	16.7
Amoxicillin 30	12.0	35.7	0.0	0.0
Augmentin30	25.0	50.0	33.3	0.0
Gentamicin10	12.0	14.3	0.0	33.4
Pefloxacin10	0.0	42.9	0.0	33.4
Tarvid 30	31.3	28.8	38.3	0.0
Streptomycin 10	31.3	14.3	33.3	16.7

Table 3 shows the Antibiotic resistance pattern of the bacterial species isolated from Kunun drinks sold in Rumuolumeni, Port Harcourt metropolis. *E. coli* exhibited very high percentage resistance to chloramphenicol (75.0%) followed by Septtrin (68.7%) and Sparfloxacin (68.7%) respectively, whereas there was no resistance to Perfloxacin (0.0%). The highest percentage resistance for *Enterobacter aerogenes* was recorded with Augmentin (50.0%) and the least resistance was recorded with Ciprofloxacin (7.1%). The percentage resistance Streptococcus spp. isolated was relatively low which ranged from 38.3% to 33.3% for the antibiotics to which this species showed resistance (Trivid, Sparfloxacin, Ciprofloxacin, Augmentin and Streptomycin). However, the isolates of the *Streptococcus* spp. showed completely no resistance (0.0%) to Septtrin, Chloramphenicol, Amoxicillin, Gentamicin and Perfloxacin. Similarly, the percentage resistance of *Staphylococcus aureus* isolated was relatively low which ranged from 33.4% to 16.7% for the antibiotics to which these isolates showed resistance (Gentamicin, Perfloxacin, Septtrin, Sparfloxacin, Ciprofloxacin and Streptomycin).

IV. DISCUSSION

The relative high numbers of microbial counts obtained from the different samples of kunu in the study were indicative of high level of microbial contamination of the prpduct. The Kunu sold at Town Hall had the highest counts of 8.6×10^2 cfu/ml, while the one from St Johns location had the lowest counts of 4.0×10^2 cfu/ml. The high microbial counts experienced may be attributed to lack of effective precautions on hygiene practice in handling procedures during processing of the beverage. The practice of addition of some quantity

of water to Kunu after fermentation may also be a source of microbial contaminants, which may have come from the water itself or from the utensils used for such purposes. In an earlier report, Amusa and Ashwaye (2009) had stated that the presence of coliforms such as *Escherichia coli* in hawked Kunu was as a result of contaminated water, containers, as well as dirty environment where the Kunu were being processed and hawked. The identification of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp and *Enterobacter aerogenes* in the samples analyzed is a positive sign to the fact that the Kunu drink sold in the different locations in the community was contaminated with potentially pathogenic bacteria and this may have come from the water used for domestic purposes, or the human handlers during processing and sales of the product, respectively. This is in agreement with Amusa and Ashwaye (2009) and Akoma *et al.*, (2013), who had noted that water used for production coupled with the crude method of production and packaging under improper sanitary conditions predisposes Kunudrink to microbial contamination of both gram negative and gram positive bacteria. The source of contamination may also have come from the spices used additives (Essien *et al.*, 2009, Lawal, 2012). There is therefore need for surveillance by Public Health officials to ensure safety of the Kunu sold for to public. There is need to also ensure that the water used for production especially post-heating processing of the Kunu is safe and free from microbial contaminants.

Antibiogram of the isolates revealed varying levels of resistance to the antibiotics tested. *Escherichia coli* showed high resistance to chloramphenicol (75%), followed by Septtrin (68.7%) and Spafloxacin (68.7%), while *Enterobacter aerogenes*, *Streptococcus* spp and *Staphylococcus aureus* had low rates of resistance to all

the antibiotics tested. However, *E. coli* had very high sensitivity to Pefloxacin (100%), followed by Gentamicin (88%), Augmentin (75%), tarivid (68.7%) and Streptomycin (68.7%). *Streptococcus* spp were the most susceptible isolates which had very high sensitivity (100%) to five of the antibiotics tested, namely, Septrin, Chloramphenicol, Amoxicillin, Gentamicin and Perfloxacina, respectively. *Staphylococcus aureus* was also very sensitive (100%) to Chloramphenicol, Amoxicillin, Augmentin and Tarivid, respectively. The sensitivity of these isolates to the antibiotics used are comparable to earlier reports (Falagas *et al.*, 2010, McGeer *et al.*, 2010 & Omeke *et al.*, 2019). The prevalence of resistant strains of *E. coli*, *Enterobacter aerogenes*, *Streptococcus* spp and *Staphylococcus aureus* in Kunu is a reflection of the use and misuse of the antibiotics in the society. This is not surprising because outside the hospital environment, the general populace have access to various kinds of antibiotics at any drug store even without any prescription from a medical practitioner. The Public Health implication of this study is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated Kunun and this would lead to chemotherapeutic failures among the human consumers of this popular beverage in the Rumuolumeni, Port Harcourt metropolis.

V. CONCLUSION

The presence of resistant strains of *E. coli*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Streptococcus* spp in Kunun sold in Rumuolumeni suggests that consumption of this beverage has potential health hazard to the consumers in Rumuolumeni, Port Harcourt, Nigeria. The consumers of this popular drink are therefore at health risk, which may culminate into failures of commonly used clinical antibiotics for the treatment of the infections.

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Winnicott from then to Now and the False Self

By Valdecir de Godoy Borges

Abstract- Starting with a very small analysis of the influences of Freud (Sigmund Freud 6 May 1856 in Freiberg to 23 September 1939 in London) in the studies of psychoanalysis that were the starting point for the others (metapsychology: point of view Topic Page "Dynamic" Dynamic in conflict "Freud's studies on the switches studies of understanding its functioning and the particular external and internal actions that form the individual's psyche this initial understanding that guides all or almost every other way of conceiving of mechanism understanding these forms of internal forces of the human being Lacan said "I am Freudian you if you want to be Lacanians" curiously, we have a tendency to link studies and other things to our daily lives to aspects of our routine in the Case of Donald Woods Winnicott (Plymouth, April 7, 1896-January 28, 1971) Winnicott was a pediatrician so it is not strange that his retrieved focus is on the initial process of human development. In these developmental processes, there are many disagreement points between within the thought of psychoanalytic schools. If we think of a fact of the psychoanalyst's position, for the most part, let us say in the course of the analysis, we can say that in other schools the analyst has more of the father's position while in the Winnicottian psychoanalytic clinic the analyst for most of the course of the sessions has a mother's position.

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WINNICOTTFROMTHENTONOWANDTHEFALSESELF

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I. INTRODUCTION

Antisocial tendency is linked to the child's development period when it establishes relationships with the mother, who is defined as a good-enough mother and not-good-enough mother. When the child does not receive all the care that the mother should give, the child extrapolates this lack of care necessary for the appropriate development. Such lack, such absence, makes the child develop these anti-social characteristics, such as stealing, such as having a more introverted behavior. The anti-social process is more easily reversible, having in mind that this process, when installed in the child has some consequences such as: gradual loss of creativity, increase in irritability, bearing in mind that irritability in the child may also be associated with depression, commitment to School development and commitment to social development.

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In the case of delinquent behavior, we already have a negative evolution of the framework that started anti-social and evolved to mechanisms that the child developed in time. Dealing with these mechanisms of behavior acquired in this delinquency period is harder, as it requires a return to the cause that generated and triggered this process, noting that, according to Winnicott, it results from this lack of care offered by the mother, who was not good enough.

I also emphasize that, in the Winnicottian interpretation, delinquency is an evolution in a worse diagnosis of antisocial behavior within a more favorable plan for the patient. It is always appropriate to start treatment in the early stages of antisocial behavior thinking, in this respect, that both have the same causal root, which is the lack of something when the mother was not good enough. In the case of delinquency, it is considered a worsening of the condition, with fixation of symptoms and structures that tend to keep the individual in a pathological emotional state, being classified as having worse diagnosis and greater psychotherapeutic difficulty.

A brief philosophical question of Martin Heidegger. "the ability of the being to question itself". This existentialist philosophy would have influenced the thinking of some psychoanalysts, as it probably influenced Winnicott. "Heidegger's Thought on the Being and Winnicottian Psychoanalysis of the Maturing Happening". According to Martin Heidegger, the being is capable of self-questioning, it is capable of understanding and looking in the mirror, facing itself and analyzing itself. Heidegger calls it "there-being", a short-sighted being in the world. It is important to understand this Being in the Winnicottian view, because in the case of a Being in development, not yet having the neurological capacity to distinguish Being from the mother or anyone else, this "there-being" tends to suffer from these lacks of the not-good-enough mother and to pay a price during its lifetime for these affective and emotional exclusions.

II. BIBLIOGRAPHIC REVIEW

We will start these studies with the thought of the child's integration with itself and with the environment of which he is a part, which receives and also gives back stimuli. Such stimuli build and enable internal parts and parts of the external environment to make this being integrate and constitute itself as an individual in an internal context and an internal context.

These influences frame this being, in this case, the constitution of the EGO. I will enter, to guide my studies, into the root of all psychoanalysis. Yes, let's talk about Sigmund Freud who, at the very least, is the passion of, I will not say all, but almost all psychoanalysts. The Freudian root defines EGO as "the character of the self", which is a "precipitated of abandoned object cathexes and (...) contains the history of these object choices" (FREUD, 1923, p. 43 - 44).

"The self is formed from identifications that take the place of cathexes abandoned by the Id (FREUD, 1923, p. 64)".

About the integration processes, "An adequate environment, free from violence, drugs or extreme poverty, allows children and adolescents to develop their social, physical and psychological potentials more harmoniously, it being up to parents or guardians to provide such conditions". (WINNICOTT, 1988.) Note that it is clear that, in this period of EGO formation, the child does not have a superEGO either. In this case, people around him who have this superego role, such as father, mother, brother, etc.

It is Interesting that, for Winnicott, the formation of the self is strictly linked to the good-enough-mother and in details such as: if the mother does not pass the female part, the child will have difficulties in constituting and understanding itself as a being, that is, an integral part the constitution of the EGO; if the mother does not give him male training, the child will have difficulty dealing with the internal environment. In other words, in other theories, the EGO is the part of the psychic apparatus that interacts with the external environment and that receives impulses from this external environment that affect and interact with the psychic apparatus. We could say, in the case of the Winnicottian theory, that the mother exercises in her child and for her child, in the first moment, the superego and also the EGO. It is as if the developing being borrows this Topic, or parts of this Topic, from the mother, which in this case is the psychic apparatus. As a free thought, I'm imagining the power that the internet and video games have today on children who are very exposed to games and the internet, with multiple contents. What would be the influences of these factors, so that they can take the place, or better, make up for the absence of a good-enough mother, in the formation of the EGO?

If you allow me to be bold, the EGO is always in the external environment, the ego is always in the other subject, the EGO is made up of small parts that are in the external environment. It is up to the good-enough mother to integrate them, or to suggest integration, so that the developing being creates, from these receipts, its own self.

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mother to integrate them, or to suggest integration, so that the developing being creates, from these receipts, its own self.

III. CONCLUSION

Given the facts of studies of the good-enough mother and the not-good-enough mother, it can be concluded that the latter brings great harm, many of which are extremely damaging to the individual. It is concluded that this formation of another Self as a protective mechanism of the SELF, in the vast majority of cases, suffocates the true SELF, assuming this false Self with a false hope, in order to ensure survival of the true SELF. After a while this is always a worse prognosis, as the false Self may suffocate the true SELF, where it is no longer possible to find it in the person.

Daseins analyze ontological model

Ontology (from the Greek ontos and -logy, "logical speech"; in the ensemble, "science of being"), is the part of the metaphysics that deals with nature, reality and existence.

False and true self, authentic life and non-authentic life.

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Antibacterial Efficacy of Pap Slurry Liquor on Some Diarrhogenic Organisms

By Yusuf, Lamidi

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Abstract- The antibacterial potential of pap slurry liquor on four diarrheal associated organisms (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Shigella dysenteriae*) was screened for. The pap slurry liquor was obtained in the laboratory from a pap (made from yellow Zeamays grains) which was allowed to ferment at ambient temperature of $28 \pm 1^\circ\text{C}$ for 72 hours at relative humidity $75 \pm 5\%$ by the maize natural microflora. The in vitro screening of different concentrations (100, 90:10, 80:20, 70:30 and 60:40 v/v) of the pap slurry liquor on the test isolates that was carried out using disc diffusion method, revealed a linear relationship antibacterial activities on all the test isolates. The minimum inhibitory concentration was observed at 90:10 v/v for *E. coli* and *S. aureus* and 80:20 v/v for *S. typhi* and *S. dysenteriae* while the minimum bactericidal concentration was observed at 100v/v for *E. coli* and *S. aureus* and 90:10 v/v for *S. typhi* and *S. dysenteriae*. All the data obtained were subjected to one way analysis of variance at 0.05 significant levels using the New Duncan's Multiple Range Test. The results from this study showed the antibacterial efficacy of pap slurry on the test isolates and therefore could be used in the treatment of diarrhea caused by these selected pathogens.

Keywords: pap slurry, antibacterial, diarrheal, organisms.

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I. INTRODUCTION

Pap slurry is a fermentation product from cereals found predominantly in Southern Nigeria and is usually the first native food given directly or supplemented with other food sources to babies at weaning. Ajanaku and Oluwole (2013) reported the use of pap slurry as a weaning food in western Nigeria to supplement breastfeed. It has also been shown that pap liquor has both anti-bacterial (Adebolu *et al.*, 2007) and antifungal properties (Ogunbanwo *et al.*, 2003). It is usually prepared from fermented maize, sorghum or millet in West Africa (Akingbala *et al.*, 2012). It is a popular breakfast cereal and infant weaning food in Nigeria (Akingbala *et al.*, 2012).

In most rural communities, where they do not have access to orthodox medicine, all kinds of plants or raw materials are exploited to take care of the different health challenges they encounter. For example in some communities in the Southwest Nigeria, uncooked papslurry, which is a Nigerian fermented food made from cereal grains such as maize (*Zea mays*) is used traditionally for the relieve of stomach discomfort and diarrhoea by the rural people. Olukoya *et al.*, (2012)

when carrying out research observed that pap slurry has antibacterial activity against common diarrhoeagenic bacteria and that the presence of *Lactobacilli* in the slurry was responsible for its effect. Adebolu, (2007) in her own contribution however reported that not only the slurry but the liquor also plays a significant antibacterial activity against diarrhoeagenic bacteria and that the growth inhibitory activity was more potent than the slurry on most of the organisms tested.

Moreover, Adebolu, (2007) has observed that the fermentation duration of pap slurry plays a significant role in the growth inhibitory activity of the liquor on susceptible organisms. Furthermore, Adebolu and Adaramola (2012) observed that the mode of fermentation, whether continuous or discontinuous at every 24 h at $30 \pm 2^\circ\text{C}$, plays a significant role in the inhibition. Although, a lot of work has been done on the antibacterial activity of the slurry of pap, more is still desired so that all necessary scientific intricacies will be taken care of for its usage to be maximally exploited. This present work therefore will help to determine the factors present in the slurry of fermented maize responsible for its antibacterial activity on the selected diarrhea causing bacteria and which one of the factors is the most effective.

Bacteria are known to cause gastrointestinal infections globally. Treatment of infections caused by these organisms is difficult because most bacteria causing infections have developed resistance to most of the conventional antibiotics, and therefore there is the need to search for alternative therapy to treat infections caused by these organisms, hence the pap slurry.

II. MATERIALS AND METHODS

Materials include: conical flask, beaker, petri dishes, test tubes, distilled water, methanol, Mueller Hiltonagar (MHA), wire loop, aluminum foil, cotton wool, spatula, autoclave, incubator, weighing balance, McCartney bottles, physiological saline, Bunsen burner and pipette.

a) Sterilization of glass wares

All glass wares were thoroughly washed with detergent and rinsed with distilled water, dried in hot-air oven and then sterilized at 60°C for 1-2 hours. The work bench surface was disinfected before and after carrying out any experiment to avoid contamination and to ensure aseptic working condition.

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b) Preparation of culture media

All culture media were prepared according to manufacturer's specification. After proper dissolution the, the media was sterilized in an autoclave at 121°C for 15 minutes. The sterile medium was allowed to cool to about 45°C before dispensing into sterile petri dishes.

c) Fermentation of pap to obtain its liquor

The Yellow maize (*Zea mays*, grains) was purchased at a local market in Anyigba Kogi State, Nigeria. Using a modified method of Odunfa and Adeleye (1985), the maize grains were carefully sorted by hand picking damaged and infested grains and pebbles, after which they were washed in sterile distilled water to remove dirt. Certain grams (2 kg) of the clean maize grains were steeped in two liters sterile water which was sufficient to cover the grains to avoid contamination. The steeped maize was left at room temperature ($28 \pm 1^\circ\text{C}$) for 72 hrs, after which it was drained and washed with sterile water three times and then wet milled using a clean grinding machine. Sieving of the resulting paste was done using a clean muslin cloth and the filtrate was collected in a clean plastic container and left for 72 hrs at room temperature ($28 \pm 2^\circ\text{C}$) for spontaneous fermentation to take place. At the end of the fermentation, the liquor of the fermented maize slurry (i.e the supernatant solution) which is locally called 'Omi-ogi' was decanted into a sterile container for analysis.

d) Test organisms

The Stock cultures of the clinical isolates used for this study were obtained from Medical Laboratory, Kogi State, University Teaching Hospital, Anyigba and confirmatory tests were carried out at the Microbiology Laboratory of the same university.

e) Confirmatory test on isolates

i. Indole test

The colonies were added into peptone water and incubated for 24 hours at 37°C after which 3 drops of kova's indole reagent was added and shaken gently. A red colour development within a minute indicated a positive test.

ii. Motility test

The motility test was carried out using a glass slide and a cover slip. Vaseline gel was used to form a ring on the slide and a loopful of the fluid culture (growing on peptone water) was transferred on the cover slip. The slide was inverted over the cover slip so that it adheres to the Vaseline gel, the slide was turned quickly so that the drop does not touch the slip or Vaseline gel. It was observed under a light microscope for characteristics movement.

iii. Citrate utilization test

A broth culture of the test organism was incubated in 3ml of koser's citrate medium at 37°C for 3

days. It was checked daily for growth. Presence of blue colouration and turbidity indicated a positive test.

iv. Urease test

A tube of sterile motility-indole-urea (MIU) medium was inoculated with the colony of test organisms. An indole paper strip was placed in the neck of the MIU tube above the medium and it was incubated at 37°C overnight. Production of urease was indicated by a red-pink colour in the medium.

f) Gram staining

A heat fixed of each Organism was made after which crystal violet was applied for 1-2 minutes and washed with water. The slide was flooded with Gram's iodine for 1 minute and washed with water. The slides were held in slanting positions while absolute alcohol solution was flooded over it until the blue colouration leaves the smear; it was flushed with water and drained. The slide was then counterstained with safranin solution for 30 seconds and washed under slow running water. It was blotted and observed under a light microscope. Gram negative organisms stained red or pink colouration while Gram positive organisms stained blue.

g) Preparation of cell suspension

Using physiological saline, cell suspensions were prepared to give concentrations equivalent to McFarland No7 (2.1×10^9 cells/ml). Then, 0.01ml of organisms was used for further inoculation in further testing.

h) Preparation of liquor concentration

A 9 ml of pap slurry liquor concentration was diluted in 1ml of sterile distilled water to make a concentration of 90:10 v/v. Other concentrations (80:20, 70:30 and 60:40 v/v) were also made following the same procedure.

i) Antibacterial screening of the liquor

The surface of the MHA plate was inoculated with the test organisms. Inoculum was standardized by matching the turbidity with 0.5% McFarland standard and then with a sterile cotton swab stick, the test culture was spread evenly over the plate successively in three directions to obtain an even inoculum. The plate was allowed to gel for 3–5 min. The filter papers discs (6 mm, with average fluid uptake 18 μl) prepared were impregnated into different concentrations of the pap slurry. Commercially available ready-made antibiotic disc (cephalexin) was placed on the surface as control and filter paper disc (6mm) impregnated in sterile distilled water was used as the negative control. The plate was incubated overnight at 37°C and the zone of inhibition was measured.

j) Determination of minimum inhibitory concentration (MIC)

Tube dilution method was used in the determination of MIC. The MIC was determined for each

of the test organisms at the varying concentrations of the liquor. Each test organism was inoculated into the labeled tube by taking a loopful of the standardized bacterial suspension using a flame sterilized wire loop and was incubated at 37 °C for 24 hours.

The lowest concentration where no turbidity was observed was recorded as the MIC.

k) *Determination of minimum bactericidal concentration (MBC)*

The minimum bactericidal concentration was determined using standard method. The tubes that

showed no visible growth from the test tubes used in the determination of MIC, were sub cultured onto freshly prepared Mueller Hinton agar and incubated at 37°C for 48 hrs. The least concentration at which the organisms did not recover and grow was taken as the MBC.

l) *Data analysis*

All the data obtained were subjected to one way analysis of variance at 0.05 significant levels using the New Duncan's Multiple Range Test.

III. RESULTS

Table 1: Antibacterial effect of pap slurry liquor on the selected diarrheal associated organisms

Concentrations v/v	Mean Zones of inhibition (mm) ± SE Test organisms			
	Gram - <i>E.coli</i>	Gram - <i>S.dysenteriae</i>	Gram + <i>Staph.aureus</i>	Gram + <i>S. typhi</i>
100	15.0±0.33 ^g	12.0±0.33 ^f	8.0±0.00 ^d	10.0±1.17 ^e
90:10	13.0±0.00 ^f	10.0±1.17 ^e	6.0±1.67 ^c	9.0±1.67 ^e
80:20	7.0±1.16 ^d	8.0±1.17 ^d	3.0±0.32 ^b	7.0±2.89 ^d
70:30	5.0±0.00 ^c	4.0±1.67 ^b	0.0±0.00 ^a	5.0±0.33 ^c
60:40	3.0±1.67 ^b	3.0±0.00 ^b	0.0±0.00 ^a	4.0±0.33 ^b
CN(25µg)	16.0±0.00 ^g	20.0±0.33 ^h	23.0±0.33 ⁱ	15.0±0.67 ^g
SDW	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a

Key: CN = cephalixin SDW = Sterile distilled water

Each value is the mean of three replicates, mean with the same letter are not significantly different

(P>0.05) from each other, using New Duncan's Multiple Range Test.

Table 2: Minimum inhibitory concentration of the pap slurry liquor on the test organisms

Concentration v/v	Test of organisms			
	<i>E.coli</i>	<i>S. dysenteriae</i>	<i>Staph.aureus</i>	<i>S.typhii</i>
100	-	-	-	-
90:10	-	-	-	-
80:20	+	-	+	-
70:30	+	+	+	+
60:40	+	+	+	+

Key:

- = no growth recorded.

+ = growth recorded

Table 3: Minimum bactericidal concentration of the pap slurry liquor against the test organisms

Test organisms	MBC v/v
<i>E.coli</i>	100
<i>Salmonella typhii</i>	90:10
<i>Staphylococcus aureus</i>	100
<i>Shigelladysenteriae</i>	90:10

IV. DISCUSSION

Pap slurry liquor used in this study had antibacterial activities against all the test bacteria isolates at varying concentrations. A dose dependent relationship was observed. This was evident by the clear zones of inhibition produced by the liquor on the

bacteria growth (Table 1). The reports of Oyetayo and Osho (2004) and Aderiye *et al.*, (2007), showed the antibacterial properties of maize pap slurry liquor *in vitro* on some organisms. In this study, the highest inhibition was recorded against Gram-negative *E.coli* which was most susceptible to the pap slurry liquor with the maximum zone of inhibition 15 mm at 100 v/v

concentration, while the least inhibition was on *Staph. aureus* (8 mm) at the same concentration. However, no inhibition zone was recorded for *Staph. aureus* at lower concentrations used (Table 1).

It is worthy of note that the standard antibiotic cephalixin used as control was more potent on the Gram positive organisms (*S. typhi* and *Staph. aureus*) than the Gram negative ones (*E. coli* and *S. dysenteriae*). Cephalixin is a β -lactam antibiotic in the class of the first generation cephalosporin which mode of action is by disrupting the growth of the bacterial cell wall. In contrast, the pap slurry liquor is more potent on the Gram negative organisms than the Gram positive ones. This may be due to the paucity of peptidoglycan and that the lipopolysaccharide is very thin and sits within the periplasmic space. Shigellae are intracellular parasites that are often transmitted by fecal-oral route primarily by food that multiply within the villus cells of the colonic epithelium. The ability of the liquor to inhibit *Shigella* in this study is of great interest and this corroborates the use of this pap slurry liquor as alternative therapy in the treatment of dysentery. This noticeable anti-diarrhogenic efficacy of pap slurry liquor could be associated with the antagonistic effects of the organisms present in the fermented liquor. Ijabadeniyi, (2007) who worked on the microorganisms associated with ogi produced from three variety of maize was able to identify *Lactobacillus plantarum*, *Lactobacillus fermentum*, yeast and *Saccharomyces cerevisiae* in the fermented slurry. Some of these organisms have been a very good potential source of probiotics.

Minimum inhibitory concentration value recorded (Table 2) was 90:10v/v concentration for *E. coli*, and *Staph. aureus* while 80:20 v/v was recorded for *Staph. aureus*, and *S. dysenteriae*. The results of the minimum bactericidal concentration recorded (Table 3) were 100v/v for *E. coli* and *S. typhi* while 90:10v/v concentration, for *Staph. aureus*, and *S. dysenteriae*.

V. CONCLUSION

The results from this study showed that pap slurry liquor from maize (*Zea mays*) was potent against the diarrheal associated isolates tested. The findings in this study justified the use of pap slurry liquor in the treatment of diarrhea in folklore medicine and the use could be adopted as well since it is cheaper and good source of potent probiotics. However further investigation should be conducted on the maize crop to ascertain the active antimicrobial compounds and the probable mode of actions.

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Perfil Antropométrico E Estilo De Vida Dos Acadêmicos De Medicina

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Palavras-Chave: medicina, estilo de vida, antropometria, acadêmicos.

1. INTRODUÇÃO

Medicina é um dos cursos mais concorridos pelas instituições que possui uma carga horária extensa, e muitos dos estudantes dedicam-se horas semanais ao estudo fora da sala de aula (MCKERROW *et al.*, 2020), enfrentando várias avaliações e trabalhos durante o curso, gerando demandas que interferem diretamente no estilo de vida (DAS, BHATTACHARYA; CHAKRABORTY, 2020; WILF-MIRON, KAGAN; SABAN, 2021).

As mudanças drásticas nos hábitos de vida dos acadêmicos de medicina acabam acarretando no comportamento dos mesmos, podendo provocar alterações preocupantes durante a graduação ou, até mesmo, na atuação profissional (SAFAIE *et al.*, 2020; SHAO *et al.*, 2020; CHAKRABORTY, 2020).

Comportamentos esses que estão relacionadas à alimentação rica em gorduras e ao excesso de consumo de produtos industrializados, ou até mesmo, o estresse diário provocado pelos meios de transporte, a falta de sono ou tempo para atividade física e as demais burocracias imposta (ALOTAIBI *et al.*, 2020; MCKERROW *et al.*, 2020).

Compreende-se que os acadêmicos de medicina precisam garantir uma segurança nos hábitos saudáveis, e quanto mais cedo houver uma conscientização com relação ao estilo de vida, poderá mais rápido usufruir de benefícios em longo prazo (FAN *et al.*, 2020; BERMEJO; STIEGMANN, 2020). A literatura científica ressalta a importância da orientação aos estudantes de medicina para um estilo de vida mais saudável, permitindo uma conciliação com os estudos e o cuidado com a saúde (WILF-MIRON, KAGAN; SABAN, 2021; FAN *et al.*, 2020).

Os jovens anseiam por reduzir a quantidade de gordura corporal ou aumentar a quantidade de massa muscular. Deste modo, para se obter informações seguras sobre o corpo e os hábitos saudáveis adquiridos, o melhor caminho está associado à avaliação física, como por exemplo a composição corporal e somatotipo (TUR; BIBILONI, 2019).

A composição corporal pode ser dividida em dois grupos: massa magra e massa gorda, sendo possível ter um acompanhamento mais detalhado, com precisão e confiança (TUR; BIBILONI, 2019). Entretanto, com a somatotipia é possível acompanhar e detectar o desenvolvimento durante o crescimento físico. Essa característica biotipológica pode ser dividida em: ectomorfa, mesomorfa e endomorfa (SÁNCHEZ-MUÑOZ *et al.*, 2020). Tendo em consideração, a composição corporal e o somatotipo, ambos estão relacionados diretamente com a saúde, se tornando necessária para qualquer indivíduo.

Diante disso, é fundamental o acadêmico passar por uma avaliação física, para desfrutar de um estilo de vida com satisfação sobre sua saúde e aquilo que estuda ao longo da graduação (BERMEJO; STIEGMANN, 2020). Entendendo também que o estilo

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de vida deve ser acompanhado de hábitos vantajosos, uma vez que esse tema deve ser colocado entre as necessidades de saúde (MAINI, FYFE; KUMAR, 2020).

Além disso, a obtenção dos dados coletados dos acadêmicos de medicina serve como referência para a prática no ensino, podendo assim encontrar artefatos ao discutir uma melhor solução para a saúde no geral. Nesse contexto, esse estudo teve por objetivo analisar o perfil antropométrico e estilo de vida dos acadêmicos de medicina.

II. MATERIAS E MÉTODOS

Este estudo caracteriza-se como modelo de campo transversal e investigação exploratória descritiva. Os participantes foram compostos por 69 acadêmicos, sendo 45 do gênero feminino e 24 masculino, do curso de medicina da Universidade Região de Joinville/SC (Univille). A triagem dos participantes se deu por convite pessoal, tornando a escolha intencional e constituindo assim, uma amostragem por conveniência.

O primeiro instrumento de pesquisa foi aplicado o questionário do Perfil do Estilo de Vida Individual (PEVI) dos autores Nahas, Barros e Francalacci (2000, p. 56), com 15 questões fechadas (de “a” até “o”), divididas em cinco componentes (nutrição, atividade física, comportamento preventivo, relacionamento social e controle do estresse), conforme o Quadro 1.

A escala de classificação das respostas o zero [0] significa que “absolutamente não faz parte do seu estilo de vida”; um [1] “às vezes corresponde ao seu comportamento”; dois [2] “quase sempre verdadeiro ao seu comportamento”; três [3] “a afirmação é sempre verdadeira no seu dia-a-dia” ou “faz parte do seu estilo de vida”. Deste modo, é importante ressaltar que os escores 2 e 3 denotam uma conduta positiva para a questão avaliada. Contudo, os escores nos níveis 0 e 1 estabelecem um diagnóstico negativo, indicando a existência de comportamentos de risco (Tabela 2).

Quadro 1. Os componentes e suas questões

COMPONENTES	QUESTÕES
Nutrição	a- Sua alimentação diária inclui ao menos 5 porções de frutas e hortaliças
	b- Você evita ingerir alimentos gordurosos (carnes gordas, frituras) e doces
	c- Você faz 4 a 5 refeições ao dia, incluindo um bom café da manhã
Atividade Física	d- Seu lazer inclui a prática de atividades físicas (exercícios, esportes ou dança)
	e- Ao menos duas vezes por semana você realiza exercícios que envolvam força e alongamento muscular
	f- Você caminha ou pedala como meio de deslocamento e, preferencialmente, usa as escadas ao invés do elevador
Comportamento Preventivo	g- Você conhece sua pressão arterial, seus níveis de colesterol e procura controlá-los
	h- Você se abstém de fumar e ingere álcool com moderação (ou não bebe)
	i- Você respeita as normas de trânsito (como pedestre, ciclista ou motorista); usa sempre o cinto de segurança e, se dirige, nunca ingere álcool
Relacionamento Social	j- Você procura cultivar amigos e está satisfeito com seus relacionamentos
	k- Seu lazer inclui encontros com amigos, atividades em grupo, ou participação e associações ou entidades sociais
	l- Você procura ser ativo em sua comunidade, sentindo-se útil no seu ambiente Social
Controle do Estresse	m- Você reserva tempo (ao menos 5 minutos) todos os dias para relaxar
	n- Você mantém uma discussão sem alterar-se, mesmo quando contrariado
	o- Você equilibra o tempo dedicado ao trabalho com o tempo dedicado ao lazer

Optou-se também pela coletados dos dados de dobras cutâneas através do uso de um plicômetro científico da marca Cescorf com precisão de 1mm; Estatura com a utilização de um estadiômetro de dois metros de comprimento de trena da marca Cescorf com precisão de 1mm; Peso corporal total com a utilização de uma balança digital marca Tanita com precisão de 100g; Diâmetros ósseos com a utilização de um paquímetro antropométrico da marca Cescorf com precisão de 1mm e Circunferências com uma trena de metal de 0,7mm de largura, flexível e com precisão de 1mm.

Os locais padronizados para medições são: Diâmetros (bi-epicondiliano do úmero e bi-epicondiliano do fêmur), Dobras cutâneas (supra-espinal, subescapular, tríceps, supra-ílica, panturrilha medial, axilar média e coxa) e Circunferências (do braço e da perna). Todas as coletas foram realizadas do lado direito do avaliado, respeitando as recomendações gerais dos protocolos.

Para determinação do percentual de gordura corporal foram utilizados os protocolos de Petroski (1995) para densidade corporal e Siri (1961). Utilizou-se o padrão de Lohman (1992, p. 80) para a classificação em relação à saúde, sendo a “Média” para homens

15% e para mulheres 23%, “Abaixo da Média” para homens é de 6-14% e para mulheres 9-22% e “Acima da Média” para homens é de 16-24% e para mulheres 24-31%.

O somatotipo foi elaborado conforme Heath-Carter, “*Anthropometric Somatotype Manual*” (CARTER, 2002), para classificação do tipo físico da amostra.

Antes de iniciar os procedimentos para as aplicações de ambos os instrumentos, no primeiro momento, foi feita uma reunião com os acadêmicos de medicina, em sala de aula, onde os mesmos foram informados sobre o objetivo e o que se espera com os resultados da pesquisa. Ao confirmarem, foram entregues Termo de Consentimento Livre e Esclarecido (TCLE) para assinarem e estarem cientes dos riscos e benefícios.

Os acadêmicos incluídos na pesquisa foram os que estavam matriculados no curso de medicina da Univille de Joinville/SC e que aceitaram participar da pesquisa, entregando o TCLE assinado.

Devido a existência do novo coronavírus (SARS-CoV-2), o isolamento social, na região de Joinville/SC iniciou no dia 16 de março. Entretanto, este estudo teve início em junho de 2021, devido às questões relativas à liberação ética, e segurança dos envolvidos. Desta maneira, para evitar a aglomeração, aqueles que optaram por participar das coletas agendaram dia e horário. Ressaltando que durante o

procedimento todos os envolvidos usaram máscara e luvas, e a todo momento foi incentivado o uso do álcool em gel.

Os dados foram coletados manualmente e transcritos utilizando a ferramenta do *Microsoft Excel® for Windows®10* e posteriormente foram transferidos para o programa *Statistical Package for the Social Sciences - IBM SPSS®*, versão 16.0. onde foram tratados inicialmente para análise de homogeneidade através do teste de *Shapiro-Wilk* onde foi detectada a normalidade dos dados e assim optando-se pelo teste de correlação de *Pearson*. Na sequência foram analisados através da estatística descritiva com as medidas de tendência central (média, mínimo, máximo e desvio padrão) e frequência (percentual).

Este estudo tem o parecer favorável do Comitê de Ética em Pesquisa com Seres Humanos da Universidade da Região de Joinville/SC - UNIVILLE, sob o número 4.731.301.

III. RESULTADOS

A amostra deste estudo foi composta por 69 acadêmicos, com 65,21% do gênero feminino e 34,79% do gênero masculino. Conforme a Tabela 1 é possível observar os valores de média e desvio padrão da idade, estatura, massa corporal e IMC dos acadêmicos de medicina.

Tabela 1. Caracterização da amostra.

Variável	\bar{X}	SD	Mín.	Máx.
Idade (anos)	20,77	3,35	17	37
Estatura (m)	1,69	0,10	1,50	1,90
Peso (kg)	63,70	12,37	45,00	97,20
IMC (kg/m ²)	22,27	2,78	18,17	29,27

\bar{X} : média, SD: desvio padrão, Mín.: mínimo, Máx.: máximo.

A Tabela 2 refere-se ao questionário de PEVI, apresentado os componentes, as respostas (número absoluto, porcentagem e moda).

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A Figura 1 está relacionada à coleta do somatotipo. Através do somatograma é possível visualizar uma distribuição dos acadêmicos avaliados.



ectomorfo", 7,25% "meso-endomorfico" e entre outras combinações que, para esse estudo, não se prevalecem. Assim, revela-se que os acadêmicos de medicina estão, predominantemente, concentrados fisicamente em Endomorfo.

Foi analisado, através da estatística descritiva, os dados ao %G dos acadêmicos de medicina, separado por gênero, encontrando a média (desvio)

geral em 21,55 (5,99), o gênero masculino em 17,97 (5,47) e feminino em 23,47 (5,39) Assim, observamos que a média do %G dos homens e das mulheres estão "Acima de Média".

No Quadro 3, é possível verificar os resultados obtidos (número absoluto e porcentagem) em relação a classificação do %G dos gêneros.

Quadro 3: Classificação do percentual de gordura dos gêneros.

Classificação	Homens (n/%)	Mulheres (n/%)
Muito Baixa	0%	0%
Abaixo da Média	6 (25%)	18 (40%)
Média	0%	3 (7,00%)
Acima da Média	17 (71%)	22 (49%)
Muito Alto	1 (4%)	2 (4%)

De acordo com os resultados expostos no Quadro 3, é possível observar que os homens e as mulheres se encontram "Acima da Média". Embora as demais classificações não obtenham prevalência, é possível notar, seguidamente, que 40% das mulheres se encontram "Abaixo da Média".

Estatisticamente, foi encontrada uma correlação moderada (0,52) no gênero masculino, bem como foi encontrado uma correlação forte (0,71) no gênero feminino, entre %G associado com a IMC ($p < 0,05$).

IV. DISCUSSÃO

Este estudo teve como finalidade analisar o perfil antropométrico e estilo de vida dos acadêmicos de medicina, por virtude de que esses estudantes acabam passando por extensas demandas que podem prejudicar a vida futuramente.

Observando o resultado do IMC, de início, os acadêmicos se classificam como "peso normal". Corroborando com os estudos de Souza *et al.* (2017) e Rodrigues *et al.* (2018), onde a média foi de 23,9 kg/m² e 22,86 kg/m², respectivamente, sendo considerada, pela Organização Mundial da Saúde, como peso ideal, bem como no estudo de Jesus *et al.* (2021), que analisaram 264 acadêmicos de medicina com média de 22,55 kg/m². Em contrapartida, o estudo de Cafure *et al.* (2018), mostra que os acadêmicos de medicina obtiveram uma prevalência para "Sobrepeso", assim como o estudo de Volpe *et al.* (2019), constituída de 109 alunos, onde o gênero masculino apresentou média de IMC correspondendo ao "Sobrepeso".

Contudo, é importante salientar que a literatura deixa claro que o IMC é um cálculo internacionalmente generalista, uma vez que cada corpo se desenvolve de maneiras diferentes (DIAS *et al.*, 2020). Apesar de ser amplamente utilizado, o IMC é frequentemente criticado

por sua capacidade limitada de distinguir entre massa gorda e massa livre de gordura (CHEN *et al.*, 2019).

Nesse sentido, o IMC contrapõe com o %G, uma vez que a média dos homens é de 17,97 e o das mulheres é de 23,47, estando estando classificados "Acima da Média". À vista disso, podemos perceber que os acadêmicos estão com a saúde em risco. Estatisticamente, foi encontrada uma correlação moderada no gênero masculino e uma correlação forte no gênero feminino, entre %G associado com a IMC ($p < 0,05$). Podemos deduzir que embora o IMC tenha gerado uma normalidade para essa classificação, ainda se torna universalmente ampla, e por isso sucedeu uma correlação com o %G que revela o valor, notadamente, da gordura dos participantes.

O estudo de Cafure *et al.* (2018) confirma que os acadêmicos de medicina, participantes da pesquisa, obtiveram uma prevalência para sobrepeso. Validando com estudo de Casado *et al.* (2021) que conclui que os estudantes da área da saúde possuem excesso de tecido adiposo (82,7%), de acordo com os parâmetros adotados.

Ao analisar os dados do somatotipo e comparar com o %G, podemos entender, através da literatura científica, que o indivíduo considerado Endomorfo, apresenta características como o arredondamento das curvas corporais, onde o relevo muscular é pouco notado, grande volume abdominal, pescoço curto e ombros quadrados (KRZYKAŁA *et al.*, 2020; CAMPA *et al.*, 2020). Tendo os acadêmicos do presente estudos com risco de sobrepeso ou obesidade.

Casado *et al.* (2021) salienta que os acadêmicos da área da saúde, cuidarão da população, por isso, além de compreender sobre as principais prevenções, é preciso também cuidar da própria saúde (CASADO *et al.*, 2021).

Ao investigar os resultados do presente estudo, podemos ver que o perfil dos acadêmicos contam com

indicadores positivos. Bühner *et al.* (2019), em seu estudo, expõe que 43,6% dos acadêmicos estão classificados no nível "bom", concluindo que os mesmos necessitam ser orientados a adotar um estilo de vida mais saudável, que se concilie com as atividades acadêmicas. De acordo com as características do estilo de vida geral, no estudo de Jesus *et al.* (2021) foi constatado que os componentes nutrição e atividade física detêm associações e características negativas, porém, foi detectada uma classificação positiva para o componente de comportamento preventivo, relacionamento social e controle de estresse.

De forma mais detalhada, no componente "Comportamento Preventivo", os acadêmicos participantes da presente escolheram indicadores positivos. De acordo com a literatura, podemos ver que no estudo de Bühner *et al.* (2019) 68% dos estudantes relataram que não fumam e 81% ingerem bebida alcoólica moderadamente. Contudo, outros estudos relatam controvérsias, identificando e concluindo que o consumo de álcool e tabaco aumentou de forma significativa durante o curso de Medicina (GOMES *et al.*, 2019). Reforçando com estudo de Pinheiro *et al.* (2017), que entrevistou 1.035 estudantes de medicina, onde a amostra relata que logo após entrar na faculdade o consumo aumentou, principalmente entre aqueles que relataram ter fumado alguma vez na vida.

O "Relacionamento Social" encontra-se escore alto, onde os acadêmicos do presente estudo gostam sempre de conviver em grupo, assim como andam satisfeitos com seus relacionamentos. Na pesquisa de Aquino, Cardoso e Pinho (2019) foi composta por uma amostra de 121 acadêmicos. Os resultados apontam que os estudantes de medicina evitam o relacionamento (70,2%). Contudo, no estudo de Vizzotto, Jesus e Martins (2017) foi avaliado o estilo de vida dos acadêmicos, e participaram 238 jovens de duas universidades. Revelou-se que as mulheres têm mais afinidade no componente relacionamento. Assim, interpreta-se que o diálogo no cotidiano deve fazer parte da construção do relacionamento social de seres humanos, entendendo que a convivência entre as pessoas é fundamental para não ocorrer conflitos (MENEZES *et al.*, 2017).

Nos componentes: "Nutrição" e "Controle do Estresse" resultaram em indicadores positivos para tal pesquisa. Ao analisar outros estudo, vê-se que participaram da pesquisa de Bühner *et al.* (2019) 576 acadêmicos de medicina, dos quais a maior parte da amostra possui idade entre 21 e 25 anos (58,51%), sendo do sexo feminino (58,68%), observou-se que metade dos universitários apresenta dieta balanceada. No estudo de Rodrigues *et al.* (2018) os estudantes de medicina apresentam dieta rica em alimentos calóricos, e consomem alimentos saudáveis.

Com relação ao "Controle de Estresse" é possível notar que esse componente é considerado impactante em diversas dimensões do estilo de vida de um acadêmico de medicina (RIBEIRO, RAIESKI; MACHADO, 2019). No estudo de Lima *et al.* (2019) foi identificado o nível de estresse dos acadêmicos do curso de medicina. Obteve uma amostra de 35 alunos do sexto período do curso de medicina. Os resultados mostram que os hábitos de saúde, reações ao estresse e satisfações com a vida atual estão numa escala de "preocupante" (51%, 57% e 60%, respectivamente). Assim, o estudo ressalta que o nível de estresse foi muito significativo.

Por fim, no componente de "Atividade Física", duas das questões se classificaram como indicadores negativos. Corroborando com estudo de Mendes, Correia e Kock (2020), onde esses analisaram um total de 402 acadêmicos do curso de medicina, sendo 62% do sexo feminino. Os resultados revelam que o nível de atividade física foi de 41,0%, mostrando que os acadêmicos estão na faixa de baixo nível. Em contrapartida, no estudo de Vaz *et al.* (2020) participaram 116 estudantes de medicina, com média de idade (anos) 24,3, sendo 37 homens e 79 mulheres. A prática de atividade física se classifica como "frequentemente" (n=51).

Além disso, é importante salientar que a atividade física é um dos mecanismos também estudado pelos pesquisadores, como uma ferramenta benéfica para diversos tratamentos para saúde geral (MENDES, CORREIA; KOCK, 2020), sendo essa prática fundamental para encontrar o %G ideal, contribuindo não só para o físico, mas para os aspecto mental e social (BULL *et al.*, 2020).

A limitação do estudo é vista através do baixo número amostral e a falta da coleta sociodemográfica, dado esse que poderia contribuir para análise das variáveis já postas. Assim, os resultados desta pesquisa retratam apenas a referida amostra.

V. CONCLUSÃO

De acordo com as evidências encontradas, é possível constatar que embora os resultados apresentam a maioria dos componentes com indicadores positivo, o %G dos acadêmicos de medicina estão "Acima da Média" e o componente da "Atividade Física" se classifica com um indicador negativo, gerando riscos à saúde e influenciado o estilo de vida. Nesse sentido, conclui-se que é de fundamental importância os acadêmicos buscarem estratégias para um estilo de vida benéfico e acompanhar os resultados antropométricos, para não prejudicar a saúde, assim como o tempo no campus.

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Prevalence of Carbapenem Resistant *Klebsiella Pneumoniae* in North India

By Puneeta Singh, Shalabh Malik & Vandana Lal

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Methods: 3256 *Klebsiella pneumoniae* isolates enrolled in this retrospective study, covering a 9-month period from January 2021 to September 2021. It focused on analyses the prevalence and characterization of Carbapenem-resistant *K. pneumoniae* strain isolated from the diverse samples of Blood, Urine, Pus, Body fluids, and Sputum analyzed at the microbiology laboratory of the Dr. Lal Path Labs, Delhi, North India.

Keywords: carbapenem-resistant *klebsiella pneumoniae* (CRKP), carbapenem-sensitive *klebsiella pneumoniae* (CSKP), extremely drug resistant (XDR), pan drug-resistant (PDR), amikacin, tigecycline, fosfomycin.

GJMR-C Classification: NLMC Code: QW 4, WC 202



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Puneeta Singh ^α, Shalabh Malik ^σ & Vandana Lal ^ρ

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Results and Discussion: Of the total 3662 isolates of *Klebsiella pneumoniae*, 1637 (44.7%) strains were carbapenem resistant. The majority of CRKP isolates were from Blood (67.1%) followed by Pus (61.2%), Body fluids (59.4%), Urine (42.5%), Respiratory (41.8%) and genital vaginal (13.8%). In this study, it appeared that only Tigecycline could be a good choice (susceptibility of 57%), at the same time Trimethoprim/Sulfamethoxazole (TM/SXT) and amikacin might be an alternative, its susceptibility was only slightly higher than 40% and 50%, respectively. In addition, our huge sample study possible to draw definitive conclusions for Fosfomycin (susceptibility of 71%) recommended as a supplement in treating CRKP in UTIs if the organism tests as susceptible.

These data highlight the need for regular surveillance of microbial resistance in India and guide the use of antimicrobial agents to improve infection control, prevent the misuse of carbapenems that has contributed to the appearance of extremely drug-resistant (XDR), and Pan drug-resistant (PDR) isolates.

Conclusions: Our findings concluded that the CRKP existed in north India among diverse samples that can also be associated with the presence in a high-risk because we are sitting on a time bomb of XDR and PDR bacterial infections if we do not take the necessary steps in time, then we have limited or no options for treatment. Therefore, the continuous monitoring of carbapenems is necessary to prevent the national and transnational spread of these isolates, especially in the cases when the healthcare facilities are inadequate.

Keywords: carbapenem-resistant *klebsiella pneumoniae* (CRKP), carbapenem-sensitive *klebsiella pneumoniae* (CSKP), extremely drug resistant (XDR), pan drug-resistant (PDR), amikacin, tigecycline, fosfomycin.

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I. INTRODUCTION

K. pneumoniae, described by Edwin Klebs in 1875, is a gram-negative bacterium belonging to the *Enterobacteriaceae* family. It caused severe infections in critically ill patients, newborns, immunocompromised individuals or those with other risk factors in healthcare establishments [1, 3]. Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has emerged as a major nosocomial pathogen worldwide and constitutes a significant growing public health threat in developing and developed countries due to the indiscriminate consumption of carbapenem antibiotics that has accelerated the incidence of antibiotic resistance in recent years [1, 5, 10]. Carbapenem resistance is typically mediated by the production of carbapenem-hydrolyzing enzyme through the evolution of high-risk clones by acquiring, retaining, and efficiently transmitting resistance genes, and unrestricted consumption promoted the rising trend as well. Carbapenem-resistant *Enterobacteriaceae* were first described in the early 1990s, and the isolation of carbapenem resistant *K. pneumoniae* strains occurred sporadically throughout that decade [11]. Over the past few years, however, the recovery of carbapenem-resistant *Klebsiella pneumoniae* strains from diverse clinical specimens has increased at an alarming rate because carbapenems are widely used to treat infections, especially those caused by *Enterobacteriaceae*, a producer of extended-spectrum β-lactamase (ESBL).

CRKP bloodstream infections are associated with higher mortality than other infection types of CRKP and require treatments timely, especially in hematological patients. This study was used to establish a risk prediction model of CRKP in this region and seek appropriate treatment in this population. Bloodstream infections caused by CRKP increase the rate of treatment failure and death. Recent estimates suggest that attributable mortality may be as high as 44%, particularly in the setting of bacteremia, with total economic costs exceeding \$553 million annually in the United States based on current incidence [1]. Previous studies have found a crude mortality rate ranging from 44% to 33% for diverse infections caused by carbapenem-resistant *K. pneumoniae* [5, 12]. In our region, where *K. pneumoniae* represented 18% of all urinary *Enterobacterales* isolates and the second leading

cause of health care-associated UTIs, and CRKP is increasingly implicated that accounts for 42.5% of all urinary *K. pneumoniae* isolates. In line with our study, Patients in the ICU are at a risk of infections caused by carbapenem-resistant *K. pneumoniae*, which is emerging as a risk to causes various nosocomial infections, notably 40% in respiratory tract infections, 59-60% of body fluids and pus samples. Therefore, we set out this study to determine the prevalence of carbapenem resistance among clinical *K. pneumoniae* isolates originated from different sections of the hospitals, which affiliated with our lab and walk-in lab in Delhi, northern region of India.

II. MATERIAL AND METHODS

This retrospective study was performed in the Microbiology department of *Dr. Lal Path Labs* in Delhi, North India. A total of 3662 *K. pneumoniae* isolates recovered from diverse samples were correctly identified using a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany).

Antibiotic susceptibility testing done by VITEK-2 (Biomérieux) system from the pure culture of isolated colonies of the *Klebsiella pneumonia* on Bloodagar; the Gram-negative bacteria were inoculated on to N405 card, and the breakpoint (susceptible, intermediate, or resistant) was interpreted according to Enterobacterales M100-S31 provided by the Clinical and Laboratory Standards Institute (CLSI) standards. The antibiotic susceptibility tests were conducted for Ampicillin, Amoxicillin clavulanic acid, Piperacillin-tazobactam, Ciprofloxacin, Levofloxacin, Cefuroxime, Ceftriaxone, Cefepime, Cefoperazone/sulbactam, Amikacin, Gentamicin, Trimethoprim-sulfamethoxazole, Ertapenem, Meropenem, Imipenem, Fosfomycin, and Nitrofurantoin. All ASTs, except Tigecycline, were interpreted according to the criteria of Enterobacterales in the Clinical and Laboratory Standard Institute (CLSI) guideline (2021) [15]. The interpretation of Tigecycline followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2021). However, Colistin was recommended to treat CRKP infection by

EUCAST. Thus, Colistin susceptibility was not routinely tested or included in our study because of its severe side effects such as nephrotoxicity, neuromuscular blockade.

In this study, Minimum inhibitory concentration (MIC) breakpoints were used as a testing method that have to detected carbapenem-resistance, and this test can be carried out using broth micro dilutions by automated antimicrobial susceptibility testing (AST) systems (VITEK-2). CRKP was defined as isolated *Klebsiella pneumoniae* strains resistant to carbapenem agents, including ertapenem, meropenem, or imipenem. *Klebsiella pneumoniae* isolates are associated with a suspicion of carbapenemase production based on updated carbapenem breakpoints (imipenem or meropenem or ertapenem MICs according to breakpoints defined by the CLSI at least $\geq 2\mu\text{g/mL}$). ATCC700603 was used as the quality control strain for the antibiotic susceptibility tests. To avoid duplicate counts, only the first strain was included for every patient, based on the ID number.

Statistical Analysis: The analysis was done using the Statistical Module of Myla Application from bioMérieux. Age, gender, antibiotic sensitivity and resistance with MIC were included as variables in this study. Myla is a browser-based application that consolidates and covers the microbiology data into actionable information. The Statistical Reporting Module is a specific MYLA(r) computer application which allows to Manage, Configure and generate different types of Microbiology Statistical Report in few clicks."

III. RESULTS

Of the total 3662 isolates of *Klebsiella pneumonia* from the diverse clinical specimens, 1637 isolates (44.7%) were confirmed to be 100% carbapenem-resistant, in which the highest occurrence of CRKP was found in blood samples comprising (67.1%) followed by Pus (61.2%), Body fluids (59.4%), Urine (42.7%), Respiratory (41.9%) and genital vaginal (13.8%) respectively (Table 1).

Table 1: Distribution of Carbapenem-Resistant *K. pneumoniae* in diverse samples during January to September 2021.

Samples	Total <i>K. pneumoniae</i> isolates N=3662	Carbapenem resistant <i>K. pneumonia</i> (CRKP) isolates N=1637 (44.7%)
Blood	91	61 (67.1%)
Pus	165	101 (61.2%)
Body fluids	185	110 (59.4%)
Respiratory	191	80 (41.9%)
Genital vaginal	29	4 (13.8%)
Urine	3001	1281 (42.7%)

Among the total 1637 CRKP isolates, 923 (56.4%) were isolated from males, while the remaining 714 (43.6%) were from females. Among these CRKP strains, 75% of patients were generally elderly (with a

range of 51 to >65 years), followed by younger adults (10.1%), adults (9.3%) and children (5.7%), respectively (Figure 1).

Age wise prevalence of Carbapenem resistant *K. pneumoniae* in different age groups.

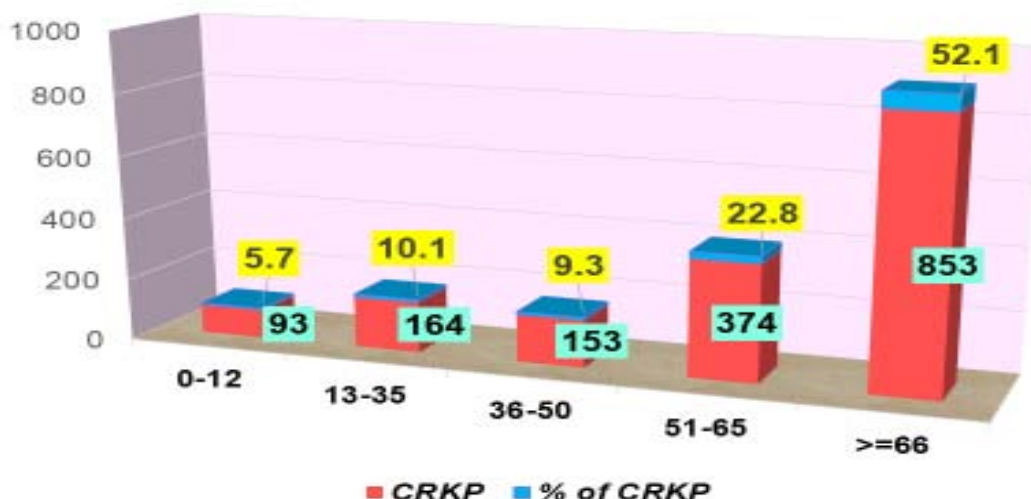


Figure 1: Age-specific distribution of Carbapenem-Resistant *Klebsiella pneumoniae* during January to September 2021.

Table 2: Percentage of Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) in diverse samples based on cumulative interpretation in different age groups during January to September 2021.

Age Specimens	0-12	13-35	36-50	51-65	≥66
Blood (n=61)	18 (29.5)	15 (24.6)	8 (13.1)	10 (16.4)	10 (16.4)
Pus (n=101)	3 (2.9)	31 (30.7)	23 (22.8)	20 (19.8)	24 (23.8)
Body Fluids (110)	2 (1.8)	22 (20)	15 (13.6)	35 (31.8)	36 (32.7)
Respiratory (80)	0	6 (7.5)	9 (11.3)	30 (37.5)	35 (43.8)
Urine (1281)	70 (5.4)	87 (6.8)	98 (7.7)	278 (21.7)	748 (57.7)
Genital (4)	0	3 (75)	0	1 (25)	0

Distribution of Carbapenem resistant *K. pneumoniae* in different age groups isolated from diverse samples during January to September 2021.

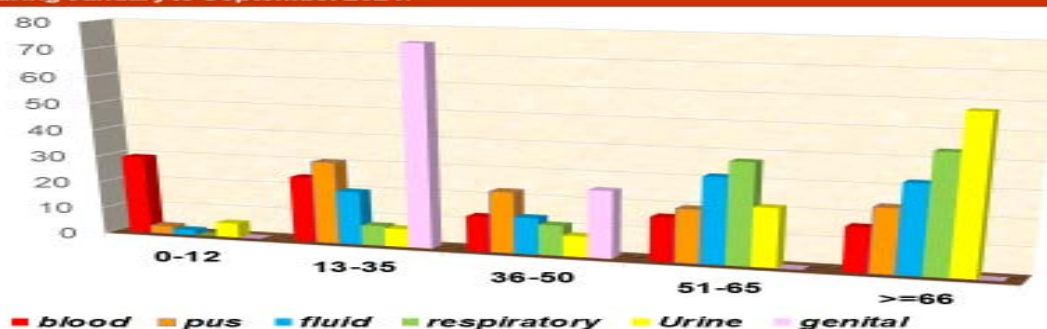


Figure 2

This study represents the prevalence of carbapenem-resistant *K. pneumoniae* in the different clinical samples in different age group has shown the maximum occurrence of CRKP in blood in children. Along with this, our study shows that the young age group (13-35) has the highest percentage of CRKP in

pus. In addition, present data reported extreme prevalence of CRKP in Body fluids, Respiratory samples is most associated in elderly people ranging from 51 to ≥66 age group while more than 50% infection with CRKP in Urine specimens associated with elderly ≥66 age group (Table 2, Figure-2).

Table 3: Antibiotic Susceptibility pattern with cumulative MIC interpretation of Carbapenem against Carbapenem Sensitive *Klebsiella pneumoniae* (CSKP) isolated from diverse samples.

Total No.	Range	CSKP N=2025 (55.3%)		CSKP MIC (µg/ml) 50/90
Antibiotics		Sensitive%	R%	
Ertapenem	≤ 0.5 - ≥ 2	97.9%	2.1%	0.5/0.5
Meropenem	≤ 1 - ≥ 4	97.2%	2.8%	<=0.25/0.25
Imipenem	≤ 1 - ≥ 4	97.6%	2.4%	<=0.25/0.5

Table 4: Antibiotic Susceptibility pattern with cumulative MIC interpretation of Carbapenem against Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) isolated from diverse samples.

Total No.	Range	CRKP N=1637 (44.7%)		CRKP MIC (µg/ml) 50/90
Antibiotics		Sensitive%	R%	
Ertapenem	≤ 0.5 - ≥ 2	0%	100	8/8
Meropenem	≤ 1 - ≥ 4	0%	100	4/16
Imipenem	≤ 1 - ≥ 4	0%	100	4/16

In this study, *K. Pneumoniae* isolates originated from different sections of the hospitals, which are affiliated with our lab and walk in the lab. This study uses current breakpoints recommended by CLSI (M100-S31) for carbapenem interpretation, 1637 (44.7%) out of 3662 *K. pneumoniae* isolates were nonsusceptible (intermediate and resistant) to Ertapenem, Meropenem and Imipenem recognized as Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP). Susceptibility pattern and their MIC value of CRKP isolates was as follows:

Ertapenem (0%; MIC_{50/90} 8ug/ml), Meropenem and Imipenem (0%; MIC_{50/90} 4 to 16ug/ml) respectively. Whereas 2025 (55.3%) recognized as Carbapenem Sensitive *Klebsiella pneumoniae* (CSKP) were susceptible to carbapenem 97% and MIC values of the tested β -lactam antibiotics were as follows: MIC_{50/90} 0.5ug/ml for Ertapenem, MIC_{50/90} <=0.25 to 0.5ug/ml for Meropenem, and Imipenem respectively in all tested strains [Table 3, 4].

Table 5: Percentage of cumulative MIC interpretation and Sensitivity of other antibiotics against Carbapenem Sensitive *Klebsiella pneumoniae* (CSKP) and Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) from all age groups during Jan to September 2021.

ORGANISM ANTIBIOTICS	Range	CSKP N=2025 (S%)	CSKP MIC (µg/ml) 50/90	CRKP N=1637 (S%)	CRKP MIC (µg/ml) 50/90
Ampicillin	≤ 8 - ≥ 32	0.9	32/32	0	32/32
Amoxycillin/clavulanic acid	≤ 8 - ≥ 32	75%	<=2/32	23%	32/32
Piperacillin/tazobactam	≤ 16 - ≥ 128	92%	<=4/16	34%	128/128
Cefuroxime	≤ 4 - ≥ 32	76%	2/64	4%	64/64
Cefuroxime/Axetil	≤ 4 - ≥ 32	73%	2/64	3%	64/64
Ceftriaxone	≤ 1 - ≥ 4	95%	1/1	6%	64/64
Cefoperazone/sulbatam	≤ 16 - ≥ 64	97%	<=8/<=8	43%	64/64
Cefepime	≤ 2 - ≥ 16	96%	1/1	35%	32/64
Amikacin	≤ 16 - ≥ 64	97%	<=2/2	54%	4/64
Gentamicin	≤ 4 - ≥ 16	96%	<=1/<=1	47%	8/16
Ciprofloxacin	≤ 0.25 - ≥ 1	62%	0.5/2	12%	4/4
Tigecycline	≤ 0.5 - ≥ 2	86%	<=0.5/2	57%	0.5/8
Trimethoprim/sulfamethoxazole	≤ 20 - ≥ 80	91%	<=20/40	42%	40/320
Fosfomycin	≤ 64 - ≥ 256	94%	<=16/64	71%	<=16/256
Nitrofurantoin	≤ 32 - ≥ 128	46%	64/128	16%	256/512

In our study, it appeared that Tigecycline was still the good choice of CRKP, with susceptibility (57%). Other options might be amikacin (54%) and TM/SXT (42%) and their MIC values were as follows: Tigecycline (57%; MIC_{50/90} 0.5/8 ug/ml), Amikacin (54%; MIC_{50/90} 4/64 ug/ml), TM/SXT (42%; MIC_{50/90} 40/320ug/ml) and Fosfomycin (71%; MIC_{50/90} 16/256ug/ml). However, the susceptibility to these antibiotics was only slightly higher than 50%. While other antibiotics sensitive pattern, which

were tested against CRKP isolates, was as follows: Amoxicillin/clavulanic acid (23%), Ceftriaxone (6%), Cefuroxime (4%), and Ciprofloxacin (12%). In recent years, Fosfomycin (susceptibility of 71%) has been recommending as a supplement in treating CRKP infection, although the CLSI standards propose it only for the treatment of urinary tract infections. The antibiogram and MIC results of the CSKP and CRKP isolates for all tested drugs are given in Table 5. Among

1637 CRKP isolates, 36(2.2%) isolates were resistant to all tested antibiotics (pan drug-resistant); these isolates did not respond to the last-resort antimicrobial Tigecycline.

IV. DISCUSSION

Klebsiella pneumoniae is among one of the most commonly detected multidrug-resistant member of the Enterobacteriales family emergence of carbapenem resistant *Klebsiella pneumonia* (CRKP) has resulted in limited effective treatment strategies, posing a healthcare threat worldwide [1-10]. The global prevalence of carbapenem-resistant *Klebsiella pneumoniae* has become alarming especially in developing and developed countries with inconsistent antibiotic policies. To our knowledge, this was the first study to focus on the burden and susceptibility of carbapenem-resistant *K. pneumonia* (CRKP) in diverse samples present alarming in north India that emphasizes contributing factor to extensive drug resistance, and their recent acquisition and dissemination likely predicted pan-drug resistance shortly. However, blood, urine, sputum, tracheal secretion and pus were the major source of CRKP worldwide, which is similar to our findings [2, 4-6, 8-10].

Our results present a worrying trend of CRKP 44.7% among *K. Pneumoniae* isolates, which is similar to the previous study where Egyptian literature showed a prevalence of 44.3% of CRKP isolates [2, 5]. Similarly, other studies showed varying prevalence rates from 20 to 60% in India and other countries [3,5 7-9]. In our study, the CRKP was found to be highest in elderly patients ranging 51 to ≥ 66 which is similar to the other studies [2, 4-6]

Our study reported that carbapenem-resistant *K. pneumoniae* was the strongest predictor of bloodstream infection among all the clinical samples studied Blood contained the highest percentage of CRKP, and this finding was also consistent with a study done in south India [10], but we have no data of mortality rates associated with severe sepsis and septic shock. Whereas other studies reported, the crude mortality rate of up to 44% attributable to carbapenem-resistant *K. pneumonia* bacteremia is the highest so far for any microorganism causing bacteremia[3]. However, other studies determined the high ratio of CRKP isolated from urine[4, 6, 7], sputum [2, 5], pus [8], respectively. In addition, the present study investigated 61% of CRKP strains were reported from different pus sites. In contrast our neighbor country reported wound samples (49.4%) were the source of the CRKP infection that were significantly associated with the general surgery ward. This study is retrospective and has some limitations, difficult to explain, such as the length of stay and time of collection of diverse samples, which could not be ascertained.

However, Urinary tract infections (UTIs) are the most common infections in India. For the first time in our region, where the emergence of carbapenem-resistant *K. pneumoniae* strains was noted that accounted for 42% of all the urinary *K. pneumoniae* isolates. In addition, particularly noteworthy findings that isolation of CRKP from the urine was most commonly associated UTIs in higher age group, which is inconsistent with other findings[4, 6-7]. Taken together, these findings provides new insights into the clinical demonstration of CRKP bacteriuria and are useful for drawing up management strategies against XDR pathogens. Our huge sample made it possible for us to draw definitive conclusions for CRKP in UTIs; Fosfomycin 71% sensitive may be a viable option for treating CRKP in Delhi if the organism tests as susceptible and we found a growing prevalence of CRKP in the UTIs through retrospective analysis, consistent with findings from previous large surveillance studies [7].

Our data showed that constantly use or misuse of carbapenems evolving Carbapenem resistance *K. pneumoniae* is the main contributing factor for XDR and usually, the definitive step before pan drug resistance (PDR) which were worrying and is dramatically limiting treatment options [13, 14]. Therefore, older agents, such as polymyxins and fosfomycin, which were rarely implemented in the past because of efficacy and toxicity concerns, together with the newer tigecycline, have become last-resort choices.

The CRKP isolates are usually XDR and are susceptible only to Tigecycline and one or more aminoglycosides. Recently, our study showed that Tigecycline, Amikacin, and TM/SXT appear to be suitable therapies for slightly higher than 50% of the bloodstream and other infections caused by carbapenem-resistant *Klebsiella pneumonia*, which draws attention to Tigecycline resistance, and this finding is similar to other studies [10, 13-14]. This is in contrast to other studies where Tigecycline had good activity against CRKP (95.5%)[2,8,12]. In this study, it appeared that only Tigecycline could be a good choice (susceptibility of 57%); at the same time, TM/SXT and amikacin might be an alternative, its susceptibility was only slightly higher than 40% and 50%, respectively. These findings indicate that the resistance rate of CRKP varies among different countries and period to period, even in the same country that may be explained in part by different levels of antibiotic use.

Therefore, we concluded that combination therapy including high-dose meropenem, fosfomycin, tigecycline and aminoglycosides are widely used, with suboptimal results is often required in the management of CRKP infections. Although there has been a need for rapid development of new antibiotics, such as ceftazidime-avibactam, and more effective counteractive measures, such as antimicrobial scientific stewardship and improved hospital infection control procedures,

have taken. These data highlight the need for regular surveillance of microbial resistance in India to improve infection control and guide for the use of antimicrobial agents. Newer BL/BLI (Beta-lactam and Beta-lactam inhibitors) are beyond the scope of this study.

V. CONCLUSION

Our findings concluded that the CRKP existed in north India among diverse isolates that can also be associated with the presence in a high-risk because we are sitting on a time bomb of XDR and PDR bacterial infections; if we do not take necessary steps like antibiotic stewardship in time, then we have limited or no options for treatment. Therefore, the importance of continuous monitoring of carbapenems that emphasizes the urgent need for improved infection control, antibiotic stewardship programs, and utilization of a surveillance and prevention system necessary to prevent the national and transnational spread of these isolates, especially in the case when the healthcare facilities are inadequate.

Ethical Approval: It is not applicable.

Conflicts of Interest: There are no conflicts of interest.

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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.



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 through 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.



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.



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:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the 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.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how 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.

THE ADMINISTRATION RULES

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CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)
BY GLOBAL JOURNALS

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	C-D	E-F
<i>Abstract</i>	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
<i>Introduction</i>	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
<i>Methods and Procedures</i>	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
<i>Discussion</i>	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
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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