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Microbiology & Pathology

Tertiary Care Hospital

Dimethoate by Cellulolytic

Highlights

Managing Thyroid Lesions

Role of the Bethesda System

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 1 VERSION 1.0

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Isolation and Partial Characterization of Virulent Phage Specific against Pseudomonas Aeruginosa

By Zahra Zahid Piracha, Umar Saeed, Aqsa Khurshid & Waqas Nasir Chaudhary

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Abstract- Pseudomonas aeruginosa is an opportunistic pathogen, frequently associated with nosocomial infections worldwide. Multiple drug resistance has been reported in previous studies against pathogenic P. aeruginosa and the biofilm which makes use of antibiotics futile. Bacteriophages specific for P. aeruginosa can prove to be a new therapeutic approach for controlling infections and biolfilm contamination against this pathogen. The aim of our study was to isolate and partially characterize virulent phage specific for P. aeruginosa from sewage water. Different parameters of which make phages as suitable candidate towards future therapeutics were also investigated. Phages having lytic life cycle, high burst size and thermally more stable proposed themselves as effective therapeutic candidates. In this study, a virulent phage was isolated from sewage water having burst size of 1036 and latent period of 21 minutes. This phage has narrow host range and shows the remarkable thermal resistance and is viable up to 60 ^{o}C.

Keywords: *pseudomonas aeruginosa, phage, isolation, partial characterization.*

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Isolation and Partial Characterization of Virulent Phage Specific against *Pseudomonas* Aeruginosa

Zahra Zahid Piracha [α], Umar Saeed [σ], Aqsa Khurshid [ρ] & Waqas Nasir Chaudhary [ω]

Abstract- ***Pseudomonas aeruginosa*** **is an opportunistic pathogen, frequently associated with nosocomial infections worldwide. Multiple drug resistance has been reported in previous studies against pathogenic *P. aeruginosa* and the biofilm which makes use of antibiotics futile. Bacteriophages specific for *P. aeruginosa* can prove to be a new therapeutic approach for controlling infections and biolfilm contamination against this pathogen. The aim of our study was to isolate and partially characterize virulent phage specific for P. aeruginosa from sewage water. Different parameters of which make phages as suitable candidate towards future therapeutics were also investigated. Phages having lytic life cycle, high burst size and thermally more stable proposed themselves as effective therapeutic candidates. In this study, a virulent phage was isolated from sewage water having burst size of 1036 and latent period of 21 minutes. This phage has narrow host range and shows the remarkable thermal resistance and is viable up to 60 ºC. Sincere efforts in term of identification, isolation, purification and characterization of multiple types of phages against *Pseudomonas aeruginosa* ; and development of cocktail with pool of lytic phages against it can prove to be a promising strategy to overcome frequent bacterial proliferation.**

Keywords: pseudomonas aeruginosa, phage, isolation, partial characterization.

I. Introduction

Pseudomonas aeruginosa is Gram negative, rod shaped opportunistic pathogen of animals, ubiquitous in nature [1]. According to United States National Nosocomial Infection Surveillance System, it accounts for 16.1% of all nosocomial infections and ranked second among Gram negative pathogens [2]. Healthy adults rarely encounter its infection but main target is people having compromised immune system including HIV infections. The infection ranges from self-limiting folliculitis to life threatening bacteremia, wound related morbidity, septicemia, skin infections, otitis media, ecthymegangrenosum, the black necrotic lesion, endocarditis, corneal ulceration and device related infections [3-4]. It is the third leading cause of 12 % of hospital-acquired urinary tract infections, upper and lower respiratory tract infections like cystic fibrosis that is associated with high mortality rate in immune-compromised patients [4]. Gender-wise prevalence showed 61.78% male and 38.22% females were infected by *P. aeruginosa* [3]. Souli and colleagues (2008) [5] published data from 23 countries on the European Antimicrobial Resistance Surveillance System and it was shown that 18% of all isolates were multidrug resistant *P. aeruginosa* strains. Aman Ullah and colleagues (2012) [6] carried out a study in Islamabad and showed that *P. aeruginosa* is 94% resistant to Chloramphenicol, 88% to Colistin /sulphate, 73% to Tetracycline and 3% to Imipenem. The resistance against the newly tested drugs is still evolving as *P. aeruginosa* is highly resistant to antibiotics, both at the genetic level and as a result of living in multilayered and complex biofilm [7].

Bacteriophages are bacterial viruses that cause lethal effect on bacteria. They have genetic material in the form of either DNA or RNA (single or double-stranded), encapsidated by a protein coat [8]. Therapeutic role of the phages was interrupted by the advent of antibiotics however the emergence of multidrug resistant bacteria and adverse effects of antibiotics led to renewed interest in phages as therapeutic agent. [9]. Phages have several advantages over antibiotics and other antimicrobial agents, such as host specificity, no side effects, and multiplication in the presence of their hosts [8]. Phages are highly specific and as they are living entity so they evolve with the evolving bacteria [10]. The specificity of phages sometimes may be considered as a possible disadvantage because there are much more pathogenic bacteria than expected to be targeted. To address this problem, a cocktail of phages should be prepared [8]. The Food and Drug Administration of the United States of America recently approved some phages as safe for food products to control Listeria infections [11]. Animal tests of phage therapy are being conducted for treatments of various bacterial infections, and many lytic phages have been isolated and tested for such applications. Phages have been used to treat the *Escherichia coli* infections and *P. aeruginosa* infections in mouse models and in guinea pigs and efficient results have been obtained [12].

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Approximately 10^30 bacteriophages are present in the environment but only about 300 phages have been characterized [13-14]. In the pre antibiotic era, phage therapy was failed because uncharacterized phages were used so in order to use them fully, it is important to isolate and characterize new phages especially in light of the observation that most of the disease-causing organisms live in matrix-enclosed environments called biofilms that inherently show increased resistance toward all antibiotics [15-16]. In the present study, we have reported the isolation and partial characterization of a virulent phage specific for *P. aeruginosa* from sewage water in Islamabad and evaluated the different parameters of phage that makes it suitable candidate for future therapeutics.

II. Materials and Methods

a) Identification of bacterial isolate

Pure cultures of bacterial strain were obtained from Microbiology laboratory of Kahuta research lab hospital, Islamabad. The bacteria were already resistant to Chloramphenicol, Colistin, Cotrimoxazole, Tetracycline and Aztreonam. After overnight incubation of bacterial strain, microbiological methods such as colony morphology and Gram staining were used for identification of bacterial strain [17].

b) Phage enrichment and isolation

Sewage water was taken from Sewage treatment plant I-9 Islamabad. Sewage water was centrifuged at 10,000 rpm for 10 min to remove algal cell and sewage debris. To enrich the phage population, above prepared sample concentrates (5 ml) were added to a 30 ml log phase *P. aeruginosa*. Enriched cultures were incubated overnight at 37 °C with shaking at 150 rpm. Chloroform (1%) was added to 1.5 ml of sample to disrupt bacterial cell and release phages and then centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was filtered using 0.45 and 0.20 mm (Minisart, SalotriusStedim Biotech) syringe filters and transferred to a new tube. Phage isolation and detection was carried out by plaque assay on LB agar plate (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, agar 15 g/L) with soft agar (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, agar 0.7%) . Overnight bacterial culture and phage sample were mixed in 0.7% LB soft agar (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, agar 0.7%; at a temperature of 45 °C) and poured over LB plates. On solidification plates were incubated overnight at 37 °C and examined for plaques the following day. For a negative control, phage alone was added to the molten agar. Well isolated plaques were serially propagated until a single phage type was obtained. The purified phages were then stored in SM buffer (100 mMNaCl, 8 mM MgSO4, 50 mM, Tris–HCl [pH 7.5]), and 0.002% w/v gelatin at 4 °C with the addition of 7% dimethyl suloxide (DMSO) at –80 °C. [18]

c) One step growth experiment

One step growth curve experiment was performed to determine latent period and burst size of phage [19-20]. In brief, 50 ml of *P. aeruginosa* culture was incubated to mid exponential phase having $O.D_{600}$ 0.6 and cells were harvester via centrifugation. The pellet was re-suspended in 0.5 ml of LB media and mixed with 0.5 ml of the phage solution having plaque forming unit (pfu) of 2.75x10^9. This mixture was allowed to stand for 3 minutes at 37 °C so that phages adsorbed to the host cells. Mixture was then centrifuged at 13,000 rpm for 2 minutes to remove the free phage particles. The pellet was re-suspended in 100 ml of LB medium and culture was incubated at 37 °C with shaking. Samples were taken after every 3 minutes for 45 minutes and after centrifugation at 13,000 rpm for 1 minute, subjected to determination of phage titer via plaque assay.

d) Analysis of calcium ion effect on phage adsorption

In order to measure the effect of divalent metal ions on phage adsorption rate $CaCl_2$ was used [21]. The 50 ml of *P. aeruginosa* overnight culture having $O.D_{600}$ 0.6 was divided in two autoclaved flask 25 ml each. One flask was inoculated with phage 500 µl having pfu of 2.75x10^9 (control), while the second flask was inoculated both with 500 µl phage (pfu 2.75x10^9) and 250 µl of 1 M $CaCl_s$ and incubated with constant shaking at 90 rpm at 37 °C. Samples were taken from both flasks at different time intervals of 0, 10, 20, and 30 minutes. Samples were centrifuged at 13,000 rpm for 3 minutes to sediment the phage adsorbed bacteria. The supernatant was assayed for unabsorbed phages via double layer plaque assay method and counts were compared with the titer of control.

e) Thermal stability analysis

Thermal stability tests were performed according to the method described by Suarez [21]. Phage filtrates were taken in microcentrifuged tubes and treated under different temperatures at 37 °C (control), 40 °C, 50 °C, 60 °C and 70 °C for 20 minutes, 40 minutes and for 1 hour. After incubation plaque assay was performed for each treated sample. Results were compared with control.

f) Bacterial reduction assay

P. aeruginosa culture 1 ml was inoculated in 2 flasks having 150 ml LB media. The phage supernatant 500 µl was introduced in one flask and other flask was treated as control and both the flasks were incubated in shaking incubator at 37 °C. The $O.D_{600}$ readings were taken after every 2 hours for 24 hours using spectrophotometer. The values were compared with control.

g) Host range

The host range of the isolated phage was checked on the range of clinical pathogenic bacterial

strains of *P. aeruginosa* isolated from blood, wound, urinary tract, ear and pus, *Streptococcus pneumoniae, Escherichia coli* and *Klebsiella pneumoniae*. All the tested bacterial strains were clinical pathogens obtained from Microbiology laboratory of Kahuta research lab hospital, Islamabad. Susceptibility of the phage was tested via the spot on lawn technique [22]. Plates were incubated upside down overnight and checked for any plaque against the negative control i.e., uninfected lawn.

III. Results

a) Identification of bacterial isolate

In this study, *P. aeruginosa* was identified via Gram staining and colony morphology. Gram staining showed that it is Gram negative bacillus.

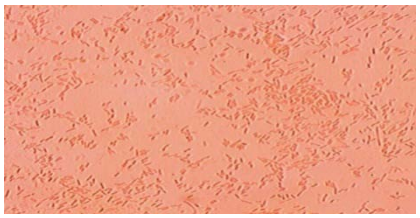

Figure 1 : Gram staining of *Pseudomonas aeruginosa* showing Gram negative bacillus rods

Table 1 : Colony morphology characteristics of *P. aeruginosa*

Sr. No	Color	Margin	Shape	Elevation	Luminous behavior	Texture	Size
1.	Off white	Undulate	Irregular	Raised, spreading edges	Translucent	Mucoid	1 mm

b) Bacteriophage isolation

P. aeroginosa was used as indicator strain for phage isolation. After enrichment, phage containing samples were placed on semisolid LB medium with *P. aeruginosa* forming lawn. Two phages were isolated that ranged from 3 to 4 mm in diameter on lawn of indicator strain by plaque analysis (Fig 2). These phages were isolated from sewage water, the same reservoir as their host. Later experiments showed that the observed plaques of two phages were similar in all aspects. Purified phage produced clear plaques on lawn of host bacterium *P. aeruginosa* (Fig 3).

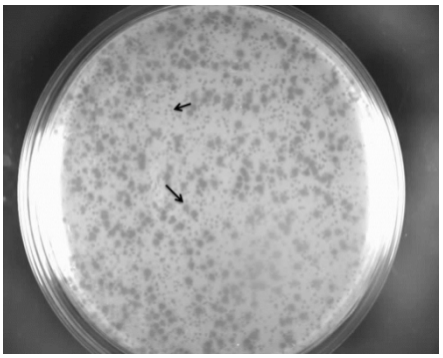

Figure 2 : Master plate of plaque assay for phage isolation from sewage water against *Pseudomonas aeruginosa*, marked points show the presence of bacterial growth clearance in the form of round zones (plaque)

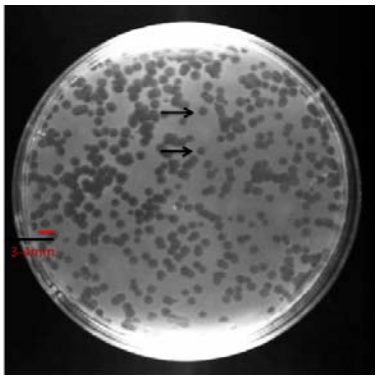

Figure 3 : Double layer agar plates showing plaque purified after repeating plaque assay three times and filtering phage lysate through 0.2 mm filter. Calculated PFU/ml was 2.75x10^9 and observed plaque size was 3-4 mm

c) One step growth experiment

One step growth curve experiment was performed to determine the latent period and burst size of the phage. Latent period is the interval between adsorption of phages to host cell and cell lysis and burst size is progeny phage particles produces by a single host bacterium was determined [19-20].The results were analyzed and the number of infective centers or constant phage titer along with the latent stage was deduced. A tri obtained including the latent phase, rise or log phase and stationery or plateau phase. The latent time of the phage was determined to be 21 minutes and burst size of the phage was 1036 phage/ infected cell. It was based on ratio of mean yield of phage particles

liberated to mean phage particle that infected the bacterial cells. There was no difference in burst size between a 1-month-old phage stock and a freshly prepared phage stock.

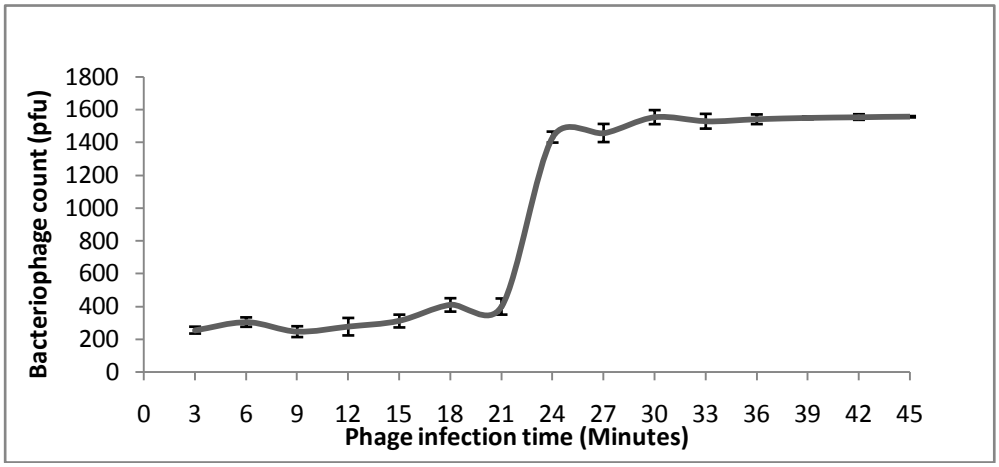

Figure 3 : One step growth curve experiment showing the latent period (21 min) and the average burst size (1036 viral particles per host cell). Latent time and burst size of phage were inferred from the curve with a tri phasic pattern. L, latent phase; R, rise phase; P, plateau phase

d) Calcium Ion Effect on the Adsorption Rate of Phage

Divalent ions often affect the adsorption of phages. In order to check this, $CaCl_2$ 1mM was added in *P. aeruginosa* phage mixture and number of free phages left in solution were detected after 10 minutes interval for 30 minutes by using plaque assay. Data analysis showed that significant differences existed between the control and calcium ion treated group. Results obtained show that calcium ions stabilize the process of adsorption and infectivity is increased due to addition of $CaCl_2$.

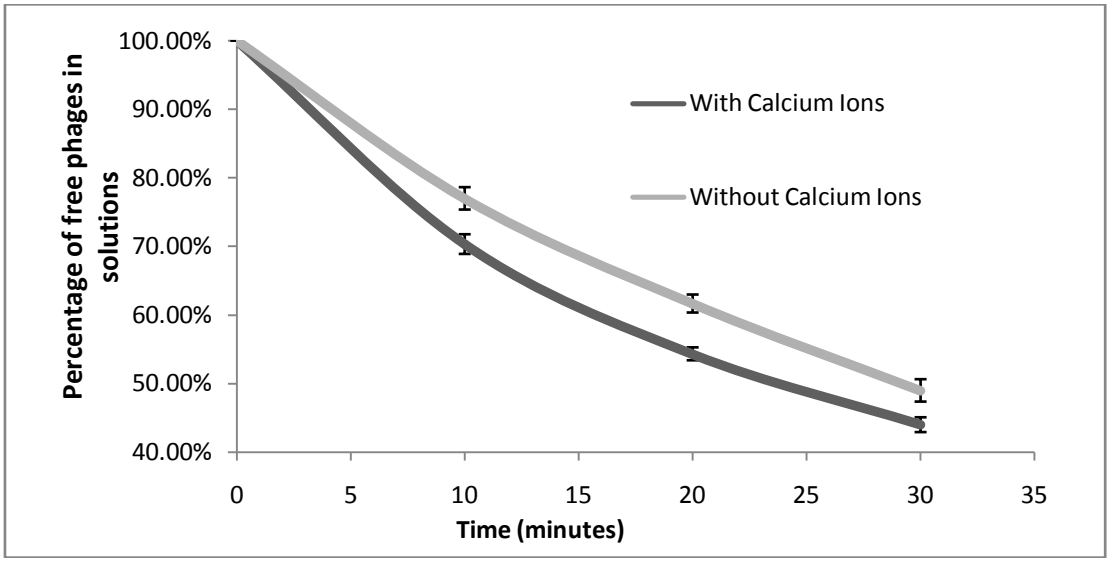

Figure 4 : Adsorption rate of phage at different time intervals shows that divalent metal ions effect on adsorption rate

e) Thermal stability analysis

Thermal stability test was carried out to check the infectivity of phage at different temperatures at pH 7.0 and also to check the resistant capability of phages. The preliminary experiments show that phages remain active at 4 °C for about 4 months (data not shown). So stability at higher temperatures was checked. Phages were amazingly stable at 35 °C, 40 °C, 50 °C and 60 °C for 20, 40 and 60 minutes. Results showed that phages are heat resistant and even 16 % phages remain viable up to 60 °C after 1 hour incubation.

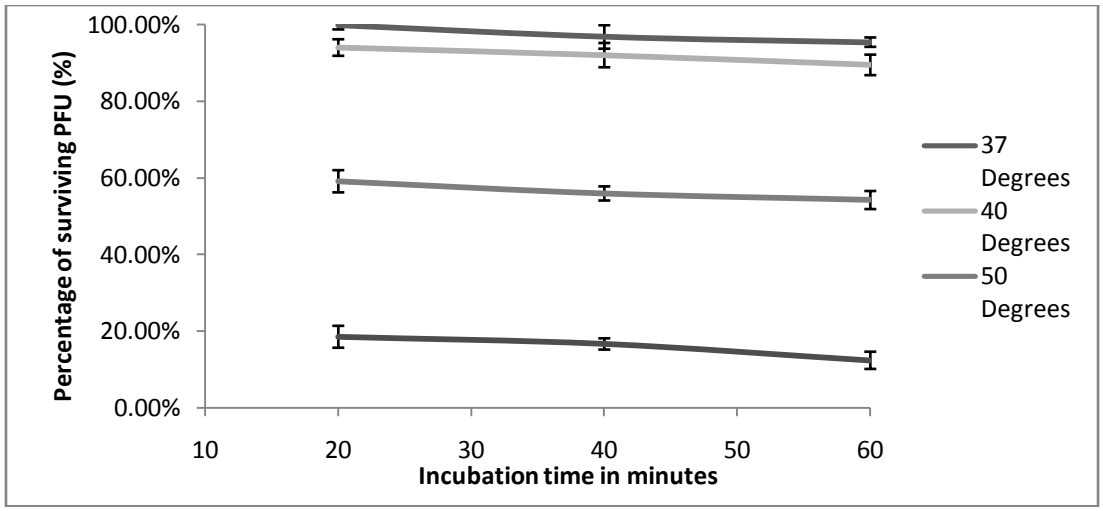

Figure 5 : Thermal stability of phages was analyzed at different temperatures and surviving phages were tittered via plaque assay

f) Bacterial reduction assay

Infection of bacteria with the phages was monitored for 24 hours. The bacterial reduction caused by phages was compared with control. Phage infection produced a drastic decrease of the *P. aeruginosa* culture as compared to control as it is evident from the graph. However, a constant increase in OD_{600} was seen after 16 hours this is most probably due to growth resistant phages.

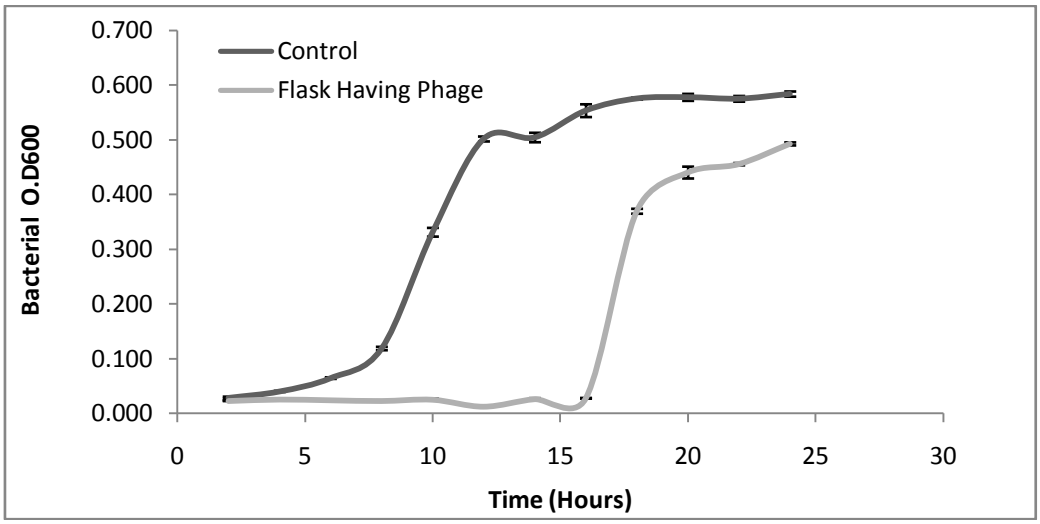

Figure 6 : The suppression and reduction of the bacterial growth by phage was compared with control. Phage infection produced a drastic decrease of the *P. aeruginosa* culture for 16 hours

g) Host range

The susceptibility of the phage was investigated with clinical strain of *P. aeruginosa* isolated from various sources and results show that the phage hasnarrow host range.

Table 2 : Host range determination of the phage

Sr. No	Source of bacteria	Clinical strain	Host range (+,-)
1.	Pus sample	*P. aeruginosa*	+
2.	Blood sample	*P. aeruginosa*	-
3.	Urinary tract sample	*P. aeruginosa*	_

4.	Wound sample	*P. aeruginosa*	+
5.	Ear sample	*P. aeruginosa*	+
6.	Ear sample	*S. pneumoniae*	-
7.	Urinary tract sample	*E. coli*	-
8.	Urinary tract sample	*K. pneumoniae*	-

IV. Discussion

P. aeruginosa is a nosocomial pathogen and leading cause of health care associated infections. It is an emerging multidrug resistant pathogen around the globe and also in Asia [2]. Way of acquiring resistance specifically the chromosome encoded efflux and low outer membrane permeability accounts for its high resistance and makes use of antibiotics futile. The biofilm induces the resistance and restricts the diffusion of the antibiotic which makes most of the antimicrobial agents less effective. It can also acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages [7]. Imipenem was considered one of the effective antibiotics however Lautenbech and colleagues in 2010 detected the imipenem resistant *P. aeruginosa* strains. All these evidence showed an alarming situation and demands an alternative treatment for *P. aeruginosa* infections [6, 23].

In this study, our results showed that a newly isolated bacteriophage can lyse actively growing *P. aeruginosa*. Phages are found with the natural habitat of their host cells such as sea water, sewage water/ sludge ponds etc [1]. They are host specific and evolve along with their host [10]. Phage was isolated from sewage water. Sewage in general, contains a large diversity of microbes due to contamination from fecal and hospital drainage water. This newly isolated phage was highly lytic and produced large plaque of 4 mm.

Phage establishes an infection in the host when it gains entry into the host after interaction with the bacterial receptor [24]. Many phages have been found to be greatly specific for their receptors present on the host cell surface. They show no interaction with receptors having a different structure. The specificity turns out to be the basis of phage typing methods used for the identification of bacterial strains. The results obtained clearly showed that our virulent phage for *P. aeruginosa* was highly specific. Results indicated that the phage may be using a common receptor.

One step growth curve explains all the stages involved in multiplication of phages. From this curve, latent period and burst size are determined. Latent period or incubation period is the time which the phage requires inside the bacterial cell and is 21 minutes in case of our isolated phage. The optimum latent period leads to high phage fitness. Rise phase is the time when infected cell begin to lyse and plateau phase is time when the infectious centers is due to only single virion [19]. Phage burst size is average number of phage bacterial cells produced per bacterial cells upon infection. The burst size of phage is 1036 particles per bacterial cell. Wang [25] described that the duration of the latent phase correlates with the burst size. The longer is the latent phage the bigger is the burst size of the phage. The number of phages liberated upon infection is quite high as compared to early reported phages.

The factors which influence the infectivity of phage in-vitro are temperature and divalent cations. The physiological state of host also plays an important role in reproduction of phage. The infection of the virion starts when it binds to host bacterial cell. In phage host interaction, calcium might be playing an important role in infection cycle [19] .Calcium ion stabilizes the weak interaction of virion with receptors or they may change the structure of the receptor to make the accessibility of phages smoothly. Ions have an electrostatic effect in the interaction of phage bacterium systems [26]. Different amount of calcium gives different infectivity.

The physical parameters like the temperature not only accelerate the adsorption of phages but also have direct effect on metabolic activities of the host [26]. Extremely thermal resistant phages have been isolated from thermal habitats and also in other aquatic environments [27]. Our results implied that phage we have isolated is active on high temperatures. The phage remained active till four months at 4 °C and it remains viable up to 60 °C after 1 hour incubation. The maximum infectivity was observed at 37 °C and least infectivity was observed at 60 °C.

The phage we isolated also causes decrease in turbidity of *P. aeruginosa* culture and this behavior was obvious for 16 hours as compared to control. There was maximum bacterial destruction during this time period but some bacterial cells show resistance to virus infection. This may be because of host bacterium adaptation as bacterial replication was going on meanwhile and bacterial resistant cells survived and though they were less in number but they started multiplying after about 16 hours and it was time when turbidity of culture increases as shown in figure 6. This

type of behavior can be a hindrance in phage therapy. Some studies shown that phage resistant bacteria losses their virulence factor because those virulence can be a site for phage infections. Such loss of virulence factor in a phage resistant bacteria mutant has been demonstrated in fish pathogens [28]. Due to resistance, the fitness of bacteria reduces and it renders it competing unfavorably with its phage sensitive ancestors. The gradual increase in optical density can also be attributed to some other factors like bacterial debris and this might be a hindrance in exposition of actual results.

V. Conclusion

Viral diseases are increasing day by day in world and it is anticipated that soon hepatitis viruses would emerge as most dangerous viral pathogens [29,30]. But on the other hand nature has selected many viruses to kill various other pathogens. . Nature has many hidden remedies against multiple disorders such as cardiovascular diseases, metabolic disorders, cancer, chronic inflammation and many others); there is strong need to discover therapeutic potentials of natural items. Many studies showed elevated activation of various cellular proteins cause cancer proliferation, which can be further inhibited by potential inhibitors (31-35). Partial characterization of the virulent phage showed that it is highly efficient in lysing *Pseudomonas aeroginosa*, as it has shown some outstanding aspects including rapid growth nature, high thermal stability and optimum latent period. All these characteristics make this phage very promising for possible application in eradication of *Pseudomonas aeroginosa* contaminations and treatment of *Pseudomonas aeroginosa* infections. Phage has shown narrow host range, so for the broad-spectrum elimination of bacteria; a "cocktail" with a pool of lytic phages might be more useful against present and other bacterial strains. A better understanding of phages and lytic enzyme biology could facilitate development of novel future therapeutics against multiple drug resistant bacteria.

References Références Referencias

1. Kenneth, V. I., Rolston, M.D., Gerald, P., Bodey, M. D., 1992. *Pseudomonas aeruginosa Infection in Cancer Patients*, Cancer Invest., 10, 43-59.
2. Ranjan, K. P., Ranjan, N., Bansal, S. K., Arora, D. R., 2010. *Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana India*, J. Lab. Physicians., 2, 74-77.
3. Khan, J. K., Iqbal, Z., Rahman, S., Farzana, K., Khan, A., 2008. *Prevalence and resistance pattern of Pseudomonas aeruginosa against various antibiotics,* Pak. J. Pharm. Sci., 21, 311-315.
4. Todar, K., 2011. *Todar's online book of bacteriology, Text book of bacteriology.net.*
5. Souli, M., Galani, I., Giamarellou, H., 2008. *Emergence of extensively drug-resistant and pandrug-resistant gram-negative bacilli in Europe,* EuroSurveil., 13, pii=19045.
6. Ullah, A., Durrani, R., Ali, G., Ahmed, S., 2012. *Prevalence of antimicrobial resistant Pseudomonas aeruginosa in fresh water spring contaminated with domestic sewage*, J. Biol. Food Sci. Res., 1, 19-22.
7. Strateva, T., Yordanov, D., 2009. *Pseudomonas aeruginosa, a phenomenon of bacterial resistance,* J. Med. Microbiol., 58, 1133-1148.
8. Clark, J. R., March, J. B., 2006. *Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials,* Trends Biotechnol., 24, 212–218.
9. Hermoso, J. A., Garcia, J. L., Garcia, P., 2007. *Taking aim on bacterial pathogens: from phage therapy to enzybiotics,* Curr. Opin. Microbiol., 10, 461–472.
10. Hausler, T., 2007. *Viruses vs. Superbugs: a solution to antibiotics or crisis? London*, Macmillan.
11. Bren, L., 2007. *Bacteria-eating virus approved as food additive,* FDA Consum., 41, 0–21.
12. Biswas, B., Adhya, S., Washart, P., Paul, B. et al., 2002. *Bacteriophage therapy rescues mice bacteremic from aclinical isolate of vancomycin-resistant Enterococcus faecium,* Inf. Immun., 70, 204–210.
13. Ashelford, K. E., Norris, S. J., Fry, J. C., Bailey, M. J., Day, M. J., 2000. *Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere,* Appl. Environ. Microbiol., 66, 4193–4199
14. Casjens, S. R., 2008. *Diversity among the tailed-bacteriophages that infect the Enterobacteriaceae,* Res. Microbiol., 159, 340–348.
15. Watnick, P., Kolter, R., 2000. *Biofilm, city of microbes,* J. Bacteriol., 182, 2675–2679.
16. Gilbert, P., Das, J., Foley, I., 1997. *Biofilm susceptibility to antimicrobials,* Adv. Dent. Res., 11, 160–167.
17. Garrity, G.M., 2005. *The Proteobacteria. Bergeys's Manaul of Systematic Bacteriology,* Springer, New York.
18. Clokie, M. R., Kropinski, A. M., 2008. *Bacteriophages: methods and protocols, volume 1: isolation, characterization and interactions,* Humana press, 501, pp. 15-21.
19. Adams, M. H., 1959. *Bacteriophage,* Interscience, New York, USA.
20. Ellis, E. L., Delbruck, M., 1939. *The growth of bacteriophage,* J. Gen. Physiol., 22, 365-384.
21. Suarez, V., Moineau, S., Reinheimer, J., Quiberoni, A., 2008. *Argentinean Lactococcuslactis bacteriophages: genetic characterization and adsorption studies.* J. Appl. Microbiol., 104, 371-379.

22. Zimmer, M., Scherer, S., Loesnner, M. J., 2002. *Genomic analysis of Clostridium perfringes bacteriophage phi 3626 which integrate into guaA and possibly affect sporulation,* J. Bacteriol., 186(16), 4359_4368.
23. Lautenbach, E., Synnestvedt, M., Weiner, M. G., Bilker, W. B. et al., 2010. *Imipenem resistance in Pseudomonas aeruginosa: emergence, epide-miology, andimpact on clinical and economic outcomes,* ICHE, 31, 47_53.
24. Richards, F., 1969. *The genetics of bacteria and their viruses,* Yale. J. Biol. Med., 42, 120–121.
25. Wang, I., Dykhuizen, D., Slobodkin, L., 1996. *The evolution of phage lysis timing,* Evol. Ecol., 10, 545–558.
26. Moldovan, R., Chapman., Quiston, M. E., Wu, X. L., 2007. *Kinetics of phage adsorption,* Bioph. J., 93, 303_315.
27. Rice, G., Stedman, K., Synder, J., Weidenheft, B. et al., 2001. *Viruses from extreme / thermal environments,* PNAS, 98, 13341-13345
28. Park, S. C., Shimarura, I., Fukunaga, M., Mori, K., Nakai, T., 2000. *Isolation of bacteriophages specific to a fish pathogen, Pseudomonas plecoglossicida, as a candidate for disease control,* Appl. Environ. Microbiol., 66, 1416–1422.
29. Saeed U, Waheed Y, Manzoor S, Ashraf M. *Identification of novel silent HIV propagation routes in Pakistan.* World J Virol 2013a; 2(3): 136-138.
30. Saeed U, Waheed Y, Ashraf M. *Hepatitis B and hepatitis C viruses: a review of viral genomes, viral induced host immune responses, genotypic distributions and worldwide epidemiology.* Asian Pac J Trop Dis 2014; 4(2): 88-96.
31. Saeed U, Jalal N. *Orange juice: a natural remedy for treatment of Cancer, prevention of chronic inflammation, Metabolic disorders and cardiov-ascular diseases.* JPHBS 2013:2;183-184.
32. Saeed U, Nawaz N, Waheed Y, Chaudry N, Bhatti HT, Urooj S. *In silico identification of BIM-1 (2-methyl-1H-indol-3-yl) as a potential therapeutic agent against elevated protein kinase C beta associated diseases.* African Journal of Biotechnology.2012;11:4434-4441.
33. Saeed U, Waheed Y, Anwar A, Hussain T, Naeem K, Nawaz N. *In-Silico Binding of ATP to Protein Kinase C Delta: Insights into the Structure and Active Site.* European Journal of Scientific Research. 2011;52: 177-187.
34. Saeed U, Jalal N, Ashraf M. *Roles of Cyclin Dependent Kinase and Cdk- Activating Kinase in Cell Cycle Regulation: Contemplation of Intracellular Interactions and Functional Characterization.* GJMR. 2012; 12:47-52.
35. Saeed U, Waheed Y, Anwar A, Ashraf M. **Structural** *Characterization of Cytochrome P450 1A2; Insights into Insilico Inhibition via Alpha- naphthoflavone.* Arch des Sci. 2013; 66: 39-50.

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Degradation of Dimethoate by Cellulolytic Bacteria in Cotton Soils

By Dr. R. Jaya Madhuri

Abstract- The present experience has been aimed to isolate bacteria specifically cellulolytic bacteria, normally found in cotton soils and determine their role in degradation of the specific pesticide. Two cellulolytic bacterial isolates k1 & k2 identified as Pseudomonas putida and Bacillus pumulus were capable of growing on dimethoate supplemented medium. P.putida exhibited maximum growth of 4.1x 106 cfu/ ml at 0.09 mg/ml while B.pumulus showed significant growth of 2.2 x107 cfu/ml at 0.06mg/ml of dimethoate after 72hrs of incubation at room temperature. Rate of utilization of dimethoate increased progressively with increase in the concentration of yeast extract added to the medium up to 0.56% in P.putida and 0.7% in B.pumulus. The present findings indicate that among the two bacterial isolates, sps of Pseudomonas could degrade 88% of dimethoate while Bacillus sps exhibited high degradation rate of 92% which can be commercialized for bioremediation of dimethoate contaminated sites. Further, the rate of degradation is maximum at 72hrs of incubation.

Keywords: *dimethoate, cellulolytic bacteria, degradation.*

GJMR-C Classification : *NLMC Code: QV 350*

Strictly as per the compliance and regulations of:

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I. Introduction

Cotton, the most important fibre crop of India plays a dominant role in its agrarian and industrial economy. It is attacked by various sucking pests-jassids, aphids, thrips, whitefly, red spider, mite and mealy bug. Bollworms such as pink bollworm, spotted bollworm and American bollworm (Tomelin, 1995).

The progressive increase of pest problem and demand for agricultural products necessitated the application of agrochemicals and ensure high quality and crop yield (Graebing et.al.,2002). Among the xenobiotics used, organophosphorous are widely applied in Indian agricultural system.

The introduction of these pesticide in to the soil environment raises concern as to their effect on ecological balance in terms of soil fertility (Balwinder Singh 2002). Even though the pesticide degradation is under the impact of various physico-chemical parameters, literature survey reveals that the major pathway governing degradation and ecotoxicity of these compounds is *microbial* mediated (Latha et al, 2001).

Dimethoate, an organophosphate broad spectrum insecticide is of particular concern as it is widely used for controlling cotton pests. But in view of its toxicity, it is important to remove dimethoate from the environment. The role of microorganisms in bioremediation is important because of their ability to degrade hazardous compounds into harmless ones. There is lack of information regarding biodegradation of dimethoate in cotton soils.

Hence the present work has been aimed to characterize the isolated cellulolytic bacteria from the cotton soil and for its ability to utilize dimethoate as the sole source of carbon and energy.

II. Materials and Methods

For the isolation of bacteria, soil samples from cotton cultivated fields were collected from three different locations in Guntur District (Narsarao pet, Sattena Palli and LAM farm). All soil samples were sieved through a 2mm screen and then used.

a) *Isolation and characterization of cellulolytic bacteria*

The three soil samples were enriched with plant debris and incubated with 37^{0}C for 15 days. After the incubation period, enriched soil samples were taken and serial dilutions (up to 10^{-6}) were made using sterile water. One ml aliquots of 10^{-4} and 10^{-5} dilutions were made to the sterile cellulose agar medium allowed to solidify. Triplicates for each dilution were maintained.

The plates were incubated at 37^{o}C for 3-5 days and were flooded with 1% aqueous solution of hexadecyltrimethyl ammonium bromide, the isolates showing a clear zone of hydrolysis around the colony were selected for further study. These isolates were streaked on cellulose agar slants and maintained as pure cultures.

Biochemical characterization of isolated bacteria was done referring to the Bergey's manual of Systematic Bacteriology and named the two bacterial isolates producing maximum zone of hydrolysis as K1 and K2

b) *Utilization of Dimethoate*

The rate of dimethoate utilization in terms of growth by the isolates K1 and K2 was determined at 37^{O}C in dimethoate (0.1 mg/ml) mixed mineral salt solution for 140 hrs.

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c) *Dimethoate degradation test*

The degradation of dimethoate was observed by bioassay method. 25 ml of MS solution was taken in 100 ml conical flask and 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21 and 0.24 mg/ml of dimethoate were taken to which the bacterial inoculum of K1 and K2 separately were added and incubated at 37^{0}C for 72 hrs and growth was studied by colony count technique.

d) *Effect of yeast extract on the utilization of dimethoate*

The effect of varying levels of yeast extract on the rate of dimethoate utilization was studied for the isolated bacteria K1 and K2. The bacterial isolates were allowed to grow for 24 hrs at 37° C in MS solution amended with dimethoate(0.1 mg/ml) plus yeast extract (MSDY) and in MS solution supplemented with yeast extract(MSY) only as control. The results were tabulated and interpreted in terms of viable CFU/ml in MS solution.

Dimethoate degradation test : Bacterial cultures efficient in degradation of dimethoate were incubated for 72 hrs and the percentage of degradation as well as metabolites formed are analysed by HPLC technique using hexane as mobile phase and C18 column at IIHR, Bangalore.

III. Results

a) *Isolation and characterization*

The K1 isolate obtained from cotton soil enriched with plant debris is a gram negative motile rod with 2.2μm length and 1.3μm width. It is aerobic and showed no spore formation. It is non spore forming, motile, catalase, oxidase and casein positive. Starch and gelatin are not hydrolyzed. IMVIC negative and nitrate is not reduced. According to Bergey's manual of classification, the K1 isolate was tentatively identified as *Pseudomonas putida* (Table 1).

The K2 isolate obtained is a gram positive rod with 3μm length and 0.6μm width. It is aerobic and a terminal ellipsoidal spore was observed. Utilization of urea, gelatin, citrate and nitrate reduction was observed. Hydrolysis of casein and starch was observed. The isolate was catalase positive and oxidase negative. Based on the above characteristics the K2 isolate was identified tentatively as *Bacillus pumilis.*

Table 1 : Morphological and biochemical characterization of K1 and K2

Name of the reaction	K1	K2
Gram reaction	Negative	Positive
Morphology	Rod	Rod
Cell length	2.2 μm	3 μm
Cell diameter	1.3 μm	μm
Pigment	Yellow	No pigmentation
Spore formation	Absent	Terminal, Ellipsoidal
Motility	Motile	Nonmotile
Starch hydrolysis	Negative	Positive
Catalase test	Positive	Positive
Gelatin hydrolysis	Negative	Positive
Oxidase test	Positive	Negative
Urease test	Negative	Positive
Casein hydrolysis	Positive	Positive
Nitrate reduction	Negative	Positive
Indole production	Negative	Negative
Voges Proskaeur	Negative	Negative
Citrate utilization	Negative	Positive

b) *Utilization of Dimethoate*

The growth curve of K1 and K2 isolates cultivated in 0.1 mg/ml of dimethoate is shown in tables 2 and 3. The K1 isolate showed its maximum growth rate after 20 hrs in MS solution (Tab 2).

The K2 isolate showed its maximum growth rate after 40 hrs in MS solution (Tab 3).

Table 2 : Growth of k_1 in mineral salt solution supplemented with 0.1mg/ml of dimethoate

Hours	CFU/ml
0	1.2x 10^5
20	3 x 10^5
40	4.5 x 10^5
60	1.2 x 10^5
80	4 x 10^4
100	8 x 10^3
120	6 x 10^3

Table 3 : Growth of k_2 in mineral salt solution supplemented with 0.1mg/ml of dimethoate

Hours	CFU/ml
0	1.5 x 10^5
20	2.3 x 10^5
40	3.8 x 10^5
60	1.2 x 10^5
80	4.1 x 10^4
100	5.8 x 10^3
120	3.4 x 10^3

c) *Dimethoate tolerance and utilization test*

Degradation of dimethoate provided as the sole carbon was studied in mineral salt solution. In K1 isolate it was found that maximum growth occurred at 0.09 mg/ml after incubation for 72 hrs at 37^{0}C (Tab. 4). The K2 isolate was found tolerating dimethoate up to 0.24mg/ml in MS solution with maximum growth at 0.06mg/ml after an incubation for 72hrs at 37^{0}C (Tab 5).

Table 4 : Utilization and tolerance level of dimethoate by k_1 isolate

Dimethoate concentration (mg/ml)	CFU/ml
0	1.5×10^4
0.03	2×10^4
0.06	$2.7x\ 10^4$
0.09	4.1×10^6
0.12	2.4×10^4
0.15	1.3×10^3
0.18	8.8×10^2
0.21	No growth
0.24	No growth

Table 5 : Utilization and tolerance level of dimethoate by k_2 isolate

Dimethoate concentration (mg/ml)	CFU/ml
0	1.3×10^4
0.03	1.9×10^4
0.06	2.2×10^7
0.09	1.1×10^5
0.12	2.3×10^4
0.15	1.8×10^3
0.18	1.4×10^3
0.21	0.8×10^2
0.24	0.2×10^2

d) Effect of Yeast Extract on the Utilization of Dimethoate

The K1 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.56% but the growth was effected at a concentration of 0.7% and above (Tab 6).

The K2 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.70% but the growth was drastically effected at a concentration of 0.84% and above (Tab 7).

Table 6 : Effect of yeast extract on k_1 utilization of dimethoate (0.1 mg/ ml) in mineral salts solution with initial inoculum of 1×10^3 CFU/ml

Conc. of yeast extract	Mineral salts medium with dimethoate	Mineral salts medium with dimethoate and yeast extract
0.14	2.1×10^4	2.9×10^4
0.28	2.8×10^4	3.5×10^5
0.42	3.5×10^5	1.7×10^6
0.56	4.7×10^5	4×10^6
0.70	3.3×10^4	3.1×10^5
0.84	2.5×10^4	5.1×10^4
0.98	1.6×10^3	2.8×10^4

Table 7 : Effect of yeast extract on k_2 utilization of dimethoate (0.1 mg/ ml) in mineral salts solution with initial inoculum of 1×10^3 CFU/ml

Conc. of yeast extract	Mineral salts medium with dimethoate	Mineral salts medium with dimethoate and yeast extract
0.14	1.5×10^4	1.9×10^4
0.28	1.9×10^4	2.5×10^5
0.42	$3.1x\ 10^4$	1.5×10^7
0.56	5×10^4	3.9×10^7
0.70	1×10^5	3.1×10^8
0.84	1.5×10^5	7.9×10^5
0.98	2.5×10^5	3.9×10^4

Table 8 : Rate of degradation of dimethoate by Bacillus pumulis K_1 isolate

Incubation time	% of degradation
24 hr	38
48 hr	51
72hr	88

Table 9 : Rate of degradation of dimethoate by Bacillus pumulis K_2 isolate

Incubation time	% of degradation
24 hr	43
48 hr	78
72 hr	92

e) Degradation of dimethoate by selected bacterial cultures

Bacterial cultures obtained by enrichment technique were tested for dimethoate degradation. In the present experiment, at the end of incubation, HPLC analysis shows that 38% degradation occurred at 24 hrs. At 48 hrs time interval, 51% dimethoate disappeared. Finally after 72 hrs,only 12% of the parent residue was detected in the sample. That means 88% of compound was degraded with respect to k_1. (Tab 8)Regarding k_2, only 85% of the parent compound was detected at the end of incubation period. Even though previous evidence indicate rapid degradation of the pesticide by soil bacteria, more than 90% of degradation rate was not reported. But in the present experiment, only trace amount of the pesticide residue was remaining after bacterial degradation indicating high efficiency of K_2 (. Morover, formation of extra peaks besides the parent compound indicate formation of intermediate products which means biotransformation of the parent insecticide. But due to non availability of compound, end product is not identified.

IV. Discussion

In the present study, two bacterial isolates K1 and K2 which are cellulolytic and capable of growth on dimethoate as a sole source of carbon and energy have been isolated from the cotton soils.

In this study the strain K1 showed maximum growth of 4.1x 10^6cfu/ml at 0.09mg/ml and strain K2 with significant growth of 2.2x10^7cfu/ml at 0.06 mg/ml. Bhadbade et al, (2002) reported that Bacillus lichen-iformis tolerated dimethoate upto the concen-tration of 2 mg/ml.

The K1 isolate showed maximum utilization of dimethoate at a concentration of 0.09 mg/ml and K2 isolate showed maximum utilization of dimethoate at a concentration of 0.06 mg/ml after 72 hrs at 37^{0}C. In a previous study Mandal et.al (2002) reported that *Bacillus licheniformis* showed a maximum utilization of dimethoate at 0.45 mg/ml

The K1 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.56% but the growth was effected at a concentration of 0.7% and above .

The K2 isolate when used for the utilization of dimethoate in the presence of yeast extract showed higher degrading activity. The rate of utilization of dimethoate increased progressively with the increase in concentration of yeast extract up to 0.70% but the growth was drastically effected at a concentration of 0.84% and above. Sharmila et al, (1992a and b) also reported earlier that rate of *microbial* degradation of parathion and other organophosphates in soil was regulated by the amount of yeast extract in the medium. Similar evidence was provided by Despande et al, (2001). Accordingly, rate of utilization of dimethoate by *Bacillus* sps progressively increased with yeast extract concentration up to 0.15%. This was further supported by experimental results of Kadam (2003) at Sreerampore. India.

Even though previous evidence indicate rapid degradation of the pesticide by soil bacteria, (Dixit and Banerjee, 2000; Fernandez etal, 2002; Zabik etal;2003).more than 90% of degradation rate was not reported. But in the present experiment, only trace amount of the pesticide residue was remaining after bacterial degradation indicating high efficiency of K_2.

References Références Referencias

1. Balwindar Singh S. (2002). Pesticide contamination of the environment in Punjab. Indian J Ecol. 29: 189-198.
2. Bhadbhaden B.J., Dhakephalker, P.K., Sarnaik, S.S., and Kanekar P.P. (2002). Plasmid-associated biodegradation of an organophosphorous pesticide by Pseudomonas mendocina. Biotechnology Letters 24: 647-650.
3. Deb Mandal, M. Mandal S and Pal NK. 2002. Evaluation of bioremediation potential of organophosphorous pesticide dimethoate 30%. EC by heavy metal and antibiotic resistance proteous vulgaris isolated from Ganges at Sreerampore. Indian Res J. Chem Environ. 6: 49-52.
4. Deshpande, N.N. Dhakephalkar, P.K., and Kaniker, P.P. (2001). Plasmid mediated dimethoate degradation in Pseudomonas aeruginosa, MCMB-427. 33-275-279.
5. Grabing, P., Frank, M. and Chib J.S. (2002). Effects of fertilizer and soil components and pesticide photolysis. J. Agric. Food Chem. 50: 7332-7339.
6. Kadam, T.A., Jadhav, V.D. and Gyananath, G. (2003). *Microbial* degradation of dimethoate by gram negative soil isolated from cotton field. Polln Res. 22: 443-445.
7. Latha, M.R., Indiarani, R. and Kamaraj, S. (2001). Biodegradation of Polluted soils. J Environ. Res. 11:27-29.
8. Metin, D. and Ferdag, K. (2001). Effect of some organophosphorous insecticides on soil microorganisms. Turk j Biol. 25;51-58.
9. Sharmila, M. Ramananad, K. and Sethunathan, N. (1992). Effect of yeast extract on the degradation of organophosphorous insecticides by soil enrichment and bacterial cultures. Can J *Microbial*., 35:1105 - 1110.
10. Subhas and Singh. D.K. (2003). Utilization of monocrotophos as phosphorous source by Pseudomonas aeruginosa F10B and Clavibacter michiganense subsp. Insidiosum SBL 11 Can J. *Microbial* / Rev. Can*Microbial* . 49: 101-109.
11. Tomlin, C. (1995). The pesticide Manual (incorporating the Agrochemicals Hand book), 10th edn British Crop Protection Council (BCPC).
12. Zaidi BR and Imam SH. 1996. Inoculation of microorganisms to enhance biodegradation of phenolic compounds in industrial wastewater. Isolation and identification of three indigenous bacterial strains. J. Gen Appl Microbiology 42;249-256.

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Diagnostic Role of the Bethesda System for Reporting Thyroid Lesions: Effective Tool for Managing Thyroid Lesions

By Dr. Vasudha M. Bhagat, Dr. Hemali J. Tailor, Dr. KumarBhargav R. Kaptan, Dr. Varsha Baladawa, Dr. Gunjan H. Prasad, & Dr. Peeyush K. Saini

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Abstract- Introduction: As Fine needle aspiration cytology (FNAC) is the primary investigation for the management of thyroid lesions, its interpretation is very crucial.The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) for clarity of communication recommends that each case should be reported in 1 of 6 general diagnostic categories facilitating communication among the managing team of doctors and leaves almost no confusion regarding the management of thyroid lesions.

Aim: To study utility of The Bethesda system of reporting thyroid cytopathology.

Materials and Method: During period of 1 year from 1st January to 31st December 2012 aspiration cytology has been carried out in 160 thyroid swellings referred to cytology department of a tertiary care hospital in Surat. Fine needle aspiration cytology was performed using mainly non-aspiration and aspiration techniques. All the cases were reported using TBSRTC. Cases were followed whenever possible.

Keywords: *FNAC, non aspiration technique, thyroid lesions, the bethesda.*

GJMR-C Classification : *NLMC Code: WK 200*

Strictly as per the compliance and regulations of:

Diagnostic Role of the Bethesda System for Reporting Thyroid Lesions: Effective Tool for Managing Thyroid Lesions

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Observations and Results: The cytological samples were assessed by qualified consultant pathologists and were categorized in category 1 to 6,six tier system according to TBSRTC criteria given by National Cancer Institute USA. Fine needle aspiration cytology analysis revealed 149 (93.12%) non-neoplastic and 11(6.88%)neoplastic lesions. Major bulks of 140 cases (87.5%) were of category II.

Conclusion: Application of TBSRTC bridges the gap in communication amongst the cytopathologist, endocrinologists, surgeons, radiologists, and other health care providers not only in the confined region but also worldwide and leaves no confusion regarding management of thyroid lesions. Few of the borderline lesions often create the confusion which are eliminated by TBSRT.

Keywords: FNAC, non aspiration technique, thyroid lesions, the bethesda.

I. Introduction

Thyroid enlargement is a common occurrence in most regions of the world including India. Being tertiary care hospital we frequently encounter such cases because southernGujarat and surrounding mountainous areas are one of the endemic goitrebelt in India.

Thyroid lesions are one of the most common lesions subjected to the cytopathology as Fine needle aspiration cytology (FNAC) is the first line investigation apart from other investigations like ultrasonography (USG), thyroid function tests, thyroid scan, and antibody levels are done subsequently to select the patients who require surgery and those that can be managed conservatively. [1, 2]

For the primaryevaluation of patients FNAC has proven to be a rapid, cost-effective, safe and reliable method of investigationlikein lesions of breast, lymph nodes and others. [3, 4] However, the success of FNAC is dependent on several important contributing influences including aspirator experience, skilful interpretation rational analysis and its application in management. Data from the Surveillance Epidemiology and End Results (SEER) registry show an increasing prevalence of differentiated thyroid cancer worldwide, [5, 6]

The increasing prevalence of thyroid cancer and improvements in the technology and resolution of ultrasound machines have led to an increasing number of cytological diagnostic procedures. [7] So being the primary investigation, interpretation and application of FNAC findings is very crucial for further management of thyroid lesions especially for ruling out need of surgery. Uniform communication amongst the cytopathologist, surgeons, endocrinologists, radiologists, and other health care providers will eliminate confusion regarding management. Few borderline thyroid lesions often create confusions regarding treatment. To eliminate such dilemma National Cancer Institute USA in 2007 conference meet was organised in Bethesda with one of the objectives beingto standardize the diagnostic terminology for the reporting of thyroid cytopathology results. The recommendations resulting from this conferenceled to the formation of The Bethesda System for ReportingThyroid Cytopathology (TBSRTC). This classificationscheme has achieved its purpose of standardization of thyroid-reporting cytopathology, as evidenced by several publications. [8]

It has been shown that the best way toimplement TBSRTC in a pathology laboratory and to

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convincethe clinicians of its validity and usefulness is to comparethe outcome data prior to and after the implementation of TBSRTC. Current study was mainly focussed to study the role of reporting system.

II. Material and Method

Study includes 160 cases of thyroid swellings patients referred to the cytopathology section of pathology department in a tertiary care hospital in southern gujarat between January 2012 and December 2012. Patient's details regarding history, clinical examination, thyroid function tests, clinical diagnosis, FNAC and histological data whenever possible were noted. The data were analysed in simple statistical tables.

All the cases of thyroid swelling subjected to FNAC were performed by cytopathologist. Prior to procedure, palpation was carried out to note the mobility of the thyroid during swallowing and the presence of any enlarged cervical lymph node. The patients were made to lie supine with their necks stretched up. A 23-24 gauge needle was used, with non-aspiration technique in most cases and very few cases with aspiration technique by a 10 ml disposable syringe. Two or more passes at different sites were made in each case. No major complications like penetration injury to the trachea, laryngeal nerve, or hematoma were recorded. Slides were prepared from aspirated material. In the case of cystic nodules, the cysts' contents wereaspirated, centrifuged, and slides made from the sediment for cytological examination. The slides were stained with MayGrunwald Giemsa (MGG), Papanicolou [PAP] stain and Haematoxylin and Eosin (H&E) and examined under light microscope. The microscopic diagnosis was interpreted under guidelines laid down by TBSRTC including categories I to VI (table 1) after taking into account of all available clinical, radiology and other data. Whenever possible, further sub typing was given.The cytological diagnosis were correlated with clinical features, thyroid function tests, subjected to histopathological examination whenever possible.

III. Results

Study includes 160cases with age range-between 5 to 70 years. Maximum cases were in 21 – 50 years of age group. Bulk of the cases were females comprising of 136 cases (85%) and 24 cases (15%) weremales and female: male ratio was 5.67:1. Long standing history of thyroid swelling was the main presenting symptom. Swelling was mainly diffuse and nodular in few cases. Symptoms like pain in the neck region, dysphagia, hoarseness of voice and cough were rare.

FNAC of 160 patients yielded the following diagnosis as depicted in Table 1.

a) Non neoplastic

Category I: The totals of 9 patients (5.63%) were diagnosed under nondiagnostic or unsatisfactory category.

Category II: It included most of the study cases with 140 cases (87.5%) of total. Age ranges with maximum number of cases were in 20-50 year age group. It consists of 'non-neoplastic' or 'negative for malignancy' cases like colloid goitre with 97 cases (69.29%), Thyroiditis with 36 cases (25.71%) and Adenomatoid goitre 7 cases (5%). All of these benign cases were just followed up and surgery was prevented.

Category III: It includes lesions which were not clearly benign or malignant. Conclusive opinion was not possible. We did not have any case diagnosed in this category.

Category IV: It includes 5 cases(3.12%) of follicular neoplasm (FN) and suspicious of follicular neoplasm (SFN).

Category V: It includes only 1 case (0.63%).

Category VI: It included 5 cases (3.12%) comprising of 2 cases each of papillary thyroid carcinomas and anaplastic carcinoma of thyroid with 1 case of medullary carcinoma of thyroid.

IV. Discussion

As in management of thyroid lesions, FNAC is the gold standard and primary investigation of choice along with other investigations like (USG) ultrason-ography examination, thyroid function tests, thyroid scan, and antibody levels are done subsequently to find out patients who require surgery and those that can be managed conservatively. [1, 2]

Being a tertiary care hospital we have many patients of thyroid disorders from the South Gujarat region including Bharuch, Vapi, Songadh, Vyara and other goitre belts. Majority of these lesions are usually benign and require no aggressive treatment. So interpretation in each case is very crucial for further management Also we want to establish uniform communication between the pathologist, radiologist, endocrinologist, surgeons and treating physicians. So that there would be no confusion regarding further management.

TBSRTC is a vital guideline which can bridge the communication gap and useful to maintain uniformity not on in the confined region but also worldwide. We followed the TBSRTC guidelines and every case was classified according to six tier reporting guideline from category I to VI.

Study includes total 160 cases of thyroid lesions which comprised of 140 cases (87.5%) of total. Published data suggest FNA has an overall accuracy rate around 75% in the detection of thyroid malignancy.[9]

The age group which was studied ranged from 5 years to 70 years and maximum no. of cases were in the age group 20-50 years means bulk of thyroid diseases were frequently encountered in young and middle aged group also the majority of cases were the females in reproductive age groups. In present study a female preponderance was noted. Similar female preponderance was noted by Unnikrishnan et al. [10]

Neoplastic lesions were 11 cases (6.88%). The benign cyst consistent with thyroglossal cyst were 4 cases (2.06%) and others were 4 cases (2.06%). The bulk of the goitre cases were in the age group of 20-50 years and thyroiditis cases were in 11-40 years and mainly in the reproductive age group of the females.

Category I: This category includes cases in which sufficient material was not available like insufficient follicular cells (Satisfactory for evaluation: six groups of well visualised follicular cells with at least ten cells per group), cyst fluid only, obscuring blood, only macrophages, preparation artefact. In such cases repeat FNA was carried out under ultrasound guidance.

Category II: Majority of lesions were benign mainly of colloid goitre 97 cases (69.29%) out of 140. In comparison to various studiesbenign category includes 60-70% [8] reason for that is we have goitre belt here. Similar findings were observed Unnikrishnan et al. [10]The chances of thyroiditis after reproductive age appeared minimal from this study.

Category III: It is reserved for specimens that contain cells (follicular, lymphoid, or other) with architectural and/or nuclear atypia that is not sufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignant. Management in such lesions is repeat FNAC after an appropriate interval. To be noted that this category is of last resort & should not be used indiscriminately. [8]

Category IV: The goal of this category is to identify all potential follicular carcinomas and refer them for a diagnostic lobectomy. Although these cytomorphologic features do not permit distinction from a follicular adenoma (FA), they are reportable as Follicular Neoplasm (FN) or suspicious of Follicular Neoplasm (SFN), leading to a definitive diagnostic procedure, usually lobectomy. [11, 12, 13] The term SFN is preferred by some laboratories over FN for this category because a significant proportion of cases (up to 35%) prove not to be neoplasms but rather hyperplastic proliferations of follicular cells, most commonly those of multinodulargoiter. [14-17]About 15% to 30% of cases called FN/SFN prove to be malignant. [11, 14, 16] The majority of FN/SFN cases turn out to be FAs or adenomatoid nodules of multinodulargoiter, both of which are more common and outnumbers the Follicular carcinoma.

Category V: FNAC can diagnose many of the thyroid cancers with fair accuracy, especially papillary thyroid carcinoma (PTC) which can be diagnosed with certainty by FNA. But the nuclear and architectural changes of some PTCs are subtle and focal. This is particularly true of the follicular variant of PTC, which can be difficult to distinguish from a benign follicular nodule. [18] Other PTCs may be incompletely sampled and yield only a small number of abnormal cells. [19] If only 1 or 2 characteristic features of PTC are present or if they are only focal and not widespread throughout the follicular cell population, or if the sample is sparsely cellular, a malignant diagnosis cannot be made with certainty. Such cases occur with some regularity, and they are best classified as suspicious for malignancy," qualified as "suspicious for papillary carcinoma." Such cases suspicious for papillary carcinoma are resected by lobectomy or thyroidectomy. Most (60%-75%) prove to be papillary carcinomas, and the rest are usually FAs. [11, 14, 16] The same general principle applies to other thyroid malignancies like medullary carcinoma and lymphoma, but these are less frequent than PTC. Such cases were considered after correlating the other findings like serum calcitonin and calcium levels and other relevant data in medullary carcinoma.

Category VI: The malignant category is used whenever the cytomorphologic features are indicative of malignancy. After confirming the malignancy the sub classification was done after summarizing all the results. Approximately 3% to 7% of thyroid FNAs have conclusive features of malignancy, and most are papillary carcinomas. [11, 14] Malignant lesions are usually treated bythyroidectomy, with some exceptions (e.g., metastatic tumors, non-Hodgkin lymphomas, and undifferentiated carcinomas). According to studies the positive predictive value of a malignant FNA interpretation is 97% to 99%.

We here practiced mainly the non-aspiration technique in almost all cases for studying cytology and found that it is better than aspiration technique. Aspiration technique is associated with low cellularity and more blood as compared to non-aspiration method. We recommend the non-aspiration method for FNAC of thyroid lesions. Similar suggestions by different studies like Maurya et.al [20] also recommend the non-aspiration technique better for thyroid lesion evaluation by FNAC.
The study found thatit is difficult to differentiate follicular/Hurthle cell adenoma from carcinoma on cytological assessment because cytology cannot evaluate the criteria of vascular or capsular invasion or of intrathyroid spread. But the papillary carcinoma, Anaplastic carcinoma and medullary carcinoma can be diagnosed by characteristic cytological features.

V. Conclusion

TBSRTC is a vital guide for accurate management of thyroid lesions. Classifying the lesions in six categories and following the guidelines given by

The Bethesda USA meetings solves all problems regarding the management of thyroid lesions and leaves no confusion. It plays a big role in establishing the uniform communications between the managing medical personnel. Marked cellularity of the smear is the problem inherent in thyroid FNAC. Increased cellularity of the smear and loss of cohesion may be present in hyperplastic/adenomatous goiter and follicular neoplasm which causes difficulty in differentiating them. This can be solved by using The Bethesda System of Reporting thyroid lesions. We experienced that The Bethesda Reporting Systemisbest for management of thyroid lesions as it gives uniform reporting system.

References Références Referencias

1. Caruso D, Mazzaferri EL. Fine needle aspiration biopsy in the management of thyroid nodules. Endocrinologist 1. 1991: 194-202.
2. Giuffrida D, Gharib H. Controversies in the management of cold, hot and occult thyroid nodules. Am J Med 1995; 99:642-50.
3. Silverman JF, West RE, Larkin EW, Park HM, Finley JL, Swanson MS et al. The role of FNAC in the rapid diagnosis and management of thyroid neoplasm. Cancer. 1986; 57: 1164-1170.
4. Kini U, Buch A, Bantwal G. Role of FNA in the medical management of minimally enlarged thyroid. DiagnCytopathol 2006; 34:196-200.
5. Ries LAG, Melbert D, Krapcho M, et al: SEER Cancer Statistics Review, 1975–2005. Bethesda, National Cancer Institute, 2007. http:// seer.cancer. gov/csr/1975_2005/
6. Chen AY, Jemal A, Ward EM: Increasing incidence of differentiated thyroid cancer in the United States, 1988–2005. Cancer 2009; 115: 3801–3807.
7. Ross DS: Predicting thyroid malignancy (editorial).J ClinEndocrinolMetabol 2006; 91:4253–4255.
8. Ali SZ, Cibas ES: The Bethesda System for Reporting Thyroid Cytopathology. Definitions, criteria and explanatory notes. New York, Springer, 2010.
9. Robinson IA, Cozens NJ. Does a joint ultrasound guided cytology clinic optimize the cytological evaluation of head and neck masses? ClinRadiol 1999; 54:312-6.
10. Unnikrishnan et al: Endocrine Society of India management guidelines for patients with thyroid nodules: A position statement, Indian J EndolMetab. 2011 Jan-Mar; 15(1): 2–8.
11. Gharib H, Goellner JR, Johnson DA. Fine-needle aspiration cytology of the thyroid: a 12-year experience with 11,000 biopsies. Clin Lab Med. 1993; 13:699-709.
12. Layfield L, Cochand-Priollet B, LiVolsi V, et al. Post thyroid FNA testing and treatment options: a synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Diagn Cytopathol.2008; 36:442-448.
13. Mazzaferri EL. NCCN thyroid carcinoma practice guidelines.Oncology. 1999; 13:391-442.
14. Yang J, Schnadig V, Logrono R, et al. Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. Cancer. 2007; 111:306-315.
15. Deveci MS, Deveci G, LiVolsi VA, et al. Fine-needle aspiration of follicular lesions of the thyroid: diagnosis and follow-up. Cytojournal. 2006; 3:9.
16. Baloch ZW, Fleisher S, LiVolsi VA, et al. Diagnosis of "follicular neoplasm": a gray zone in thyroid fine-needle aspiration cytology. DiagnCytopathol. 2002; 26:41-44.
17. Kelman AS, Rathan A, Leibowitz J, et al. Thyroid cytology and the risk of malignancy in thyroid nodules: importance of nuclear atypia in indeterminate specimens. Thyroid. 2001; 11:271-277.
18. Chung D, Ghossein RA, Lin O. Macrofollicular variant of papillary carcinoma: a potential thyroid FNA pitfall. DiagnCytopathol. 2007; 35:560-564.
19. Renshaw AA. Focal features of papillary carcinoma of the thyroid in fine-needle aspiration material are strongly associated with papillary carcinoma at resection. Am J Clin Pathol. 2002; 118:208-210.
20. Maurya et.al: Comparison of aspiration vs non-aspiration techniques in fine-needle cytology of thyroid lesions: J Cytol. April 2010, 27(2): 51–54.

Table 1 : Distribution of thyroid lesions according to age and sex after applying TBSRTC

Age in years	Male	Female	I	II	III	IV	V	VI	Total
1-10	1	1	0	2	0	0	0	0	2
11-20	2	5	0	6	0	0	0	1	7
21-30	4	43	2	44	0	1	0	0	47
31-40	11	41	4	45	0	0	1	2	52
41-50	4	27	1	25	0	3	0	2	31
51-60	1	8	0	8	0	1	0	0	9
61-70	1	11	2	10	0	0	0	0	12
Total	24 (15%)	136 (85%)	9 (5.63%)	140 (87.5%)	0 (0%)	5 (3.12%)	1 (0.63%)	5 (3.12%)	160

I-Non diagnostic or unsatisfactory, II- Benign, III- Atypia of undetermined significance or Follicular lesions of undetermined significance, IV- Follicular neoplasm or suspicious of follicular neoplasm, V- Suspicious of malignancy, VI- Malignant lesion.

Table 2 : Diagnostic categories, implied risk of malignancy & recommended clinical management accordingTBSRTC.(Modified by Ali and Cibas)[8]

Diagnostic category	Cytological diagnosis	Risk of malignancy	Clinical management
I	Non-diagnostic or Unsatisfactory	1-4 %	Repeat FNA with ultrasound guidance
II	Benign	0-3 %	Clinical follow-up
III	AUS/FLUS*	5-15 %	Repeat FNA
IV	FNS/SFN ¶	15-30 %	Surgical lobectomy
V	Suspicious of malignancy	60-75 %	Near total thyroidectomy or Surgical lobectomy
V	Malignant	97-99 %	Near total thyroidectomy

**AUS/FLUS – Atypia of undetermined significance / Follicular lesion of undetermined significance.*
¶ FNS / SFN – Follicular neoplasm / Suspicious of follicular neoplasm.

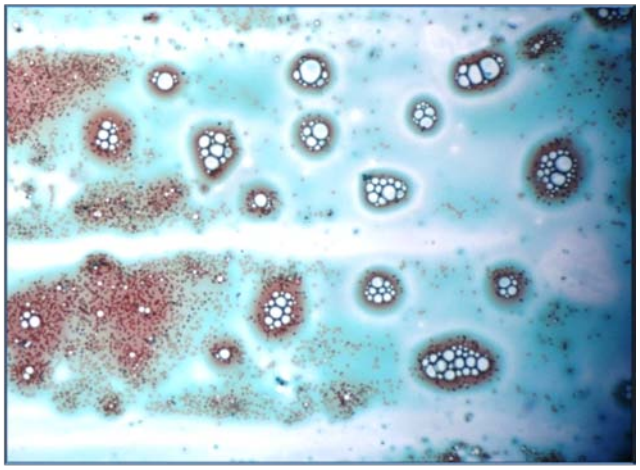

Figure 1 : Abundant watery thin colloid in Colloid Goitre

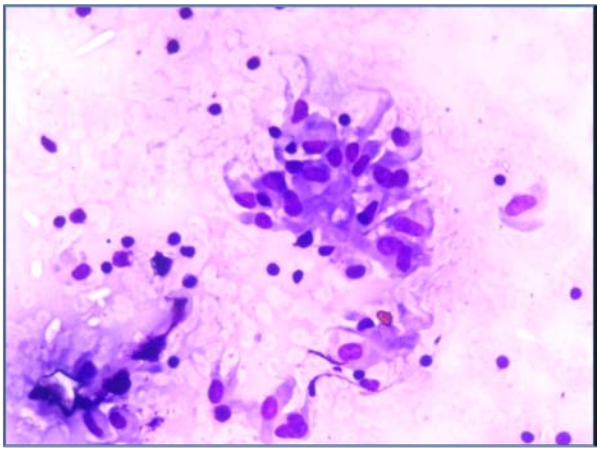

Figure 2 : Hurthle cell changes in Hashimoto thyroiditis

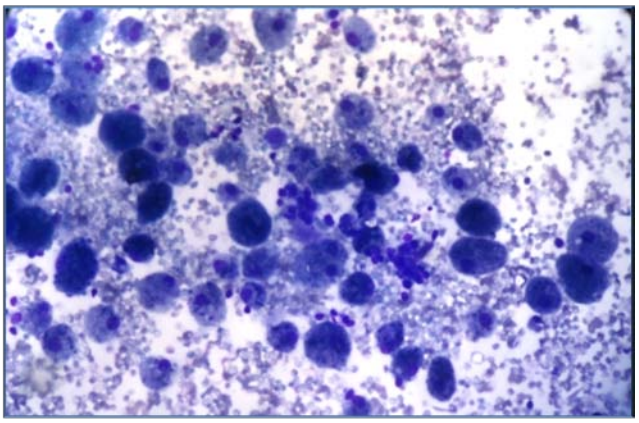

Figure 3 : Numerous macrophages only: Nondiagnostic category

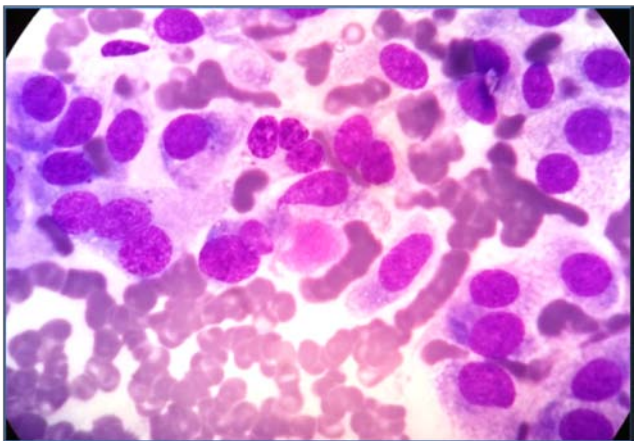

Figure 4 : Medullary carcinoma of thyroid with amyloid like material

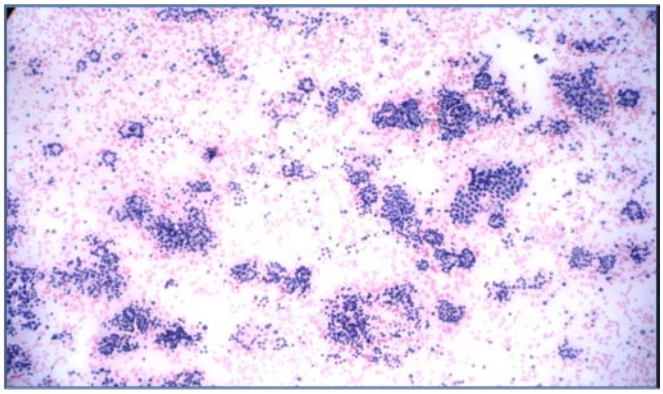

Figure 5 : Follicular neoplasm with numerous microfollicles

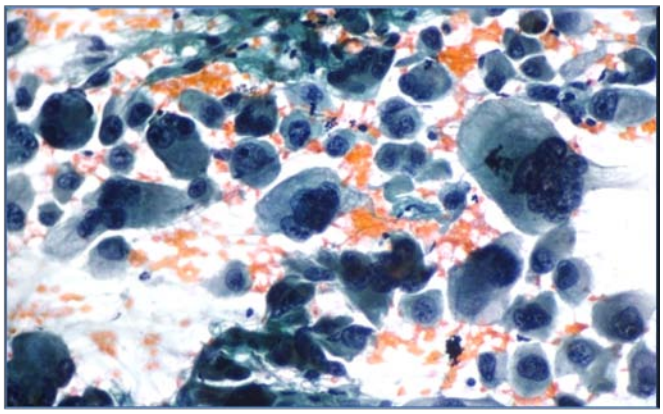

Figure 6 : Anaplstic carcinoma of thyroid

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Determination of the Causative Agents of Bacteremia in Children under 5 Years and their Susceptibility Pattern to the Antibiotics

By Dr. Abhineet Mehrotra & Dr. Shailendra Mishra
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Abstract- *Objective:* To determine the causative agents of bacteremia in children under 5 years and their susceptibility to the commonly used antibiotics.

Methods: This was a cross-sectional study on children (aged from 1 day to 5 years) admitted to the paediatric ward. The patients included all newborn babies and children admitted with fever and suspected of having sepsis. All the included children were clinically diagnosed for septicemia following strict aseptic precautions and the blood sample was taken. Blood culture were done by standard method.

Results: The overall incidence of bacteremia was 23.1%. The incidence of bacteremia was higher among the chidren of age group 13-60 month (38.1%) than <1 month (23.4%) and 1-12 month (12.5%). The male (25.8%) children were affected than females (19.6%).

Keywords: *bacteremia, incidence, children, bacterial isolates.*

GJMR-C Classification : *NLMC Code: WC 240*

Strictly as per the compliance and regulations of:

Determination of the Causative Agents of Bacteremia in Children under 5 Years and their Susceptibility Pattern to the Antibiotics

Dr. Abhineet Mehrotra [α] & Dr. Shailendra Mishra [σ]

Abstract- Objective: To determine the causative agents of bacteremia in children under 5 years and their susceptibility to the commonly used antibiotics.

Methods: This was a cross-sectional study on children (aged from 1 day to 5 years) admitted to the paediatric ward. The patients included all newborn babies and children admitted with fever and suspected of having sepsis. All the included children were clinically diagnosed for septicemia following strict aseptic precautions and the blood sample was taken. Blood culture were done by standard method.

Results: The overall incidence of bacteremia was 23.1%. The incidence of bacteremia was higher among the chidren of age group 13-60 month (38.1%) than <1 month (23.4%) and 1-12 month (12.5%). The male (25.8%) children were affected than females (19.6%). The *Klebsiella species* organism was the most common organism which was 44.4% followed by *Staphylococcus. Aureus* (14.8%) and *Coagulase Negative staphylococci (CONS)* (11.1%). The percentage of other organism was less than 10%. The percentage of gram negative bacterial isolate was among 70.4% of the samples and gram negative was 29.6%.

Conclusion: The resistance of the recovered *Klebsiella* spp. isolates to a number of antimicrobial agents was determined, and a pattern of multi-resistance was observed which may explain the prevalence of these isolates in pediatric bacteremia.

Keywords: bacteremia, incidence, children, bacterial isolates.

I. Introduction

Bacteremia is the presence of viable bacteria in the circulating blood. Bacteria may enter the blood stream giving rise to bacteremia from an existing focus of infection from a site with the commensally flora or by direct inoculation of contaminated materials into the vascular system. These organisms are often cleared from the blood within minutes, so the bacteremia is silent and transient, but if the immune system is overwhelmed or evaded, organisms persist in the blood and bacterimic symptoms would arise. Bacteremia should be distinguished from septicemia in which signs and symptoms of severe diseases are present[1]. Neonates are particularly vulnerable to infections because of their weak immune barrier. Several risk factors have been identified both in the neonates and children which makes them susceptible to infections[2]. Children with septicaemia present with fever, difficulty in breathing, tachycardia, malaise, refusal of feeds or lethargy[3].

Studies of bloodstream infections in children admitted to African hospitals suggest that the prevalence of bacterial bloodstream infections among inpatients with fever or clinical sepsis exceeds that described in wealthier regions[4,5]. Bloodstream infections continue to be the major cause of morbidity and mortality despite advance in antimicrobial therapy and supportive care[6]. Fever in infants younger than 1 year old, especially those younger than 3 months, can signal a serious infection[7].

The aim of this study was to determine the causative agents of bacteremia in children under 5 years and their susceptibility to the commonly used antibiotics.

II. Material and Methods

This was a cross-sectional study approved by the ethical committee of the institute. In this study, 117 blood samples were collected from children (aged from 1 day to 5 years) admitted to the paediatric ward of a teaching hospital in north India. The patients included all newborn babies and children admitted with fever and suspected of having sepsis. Children with fever less than 5 days and with known clinical condition such as malignancies, tuberculosis etc. were excluded from the study. The consent was taken from parent/guardian of each children before enrolling in the study.

A total of 128 children were included in this study. All the included children were clinically diagnosed for septicemia following strict aseptic precautions and the blood sample was taken. One milliliter (neonates) and 5 ml (children) blood were collected, inoculated into 10 ml and 50 ml, respectively of brain heart infusion broth. The culture bottles were incubated at 37°C aerobically and periodic subcultures were done onto Mac Conkey's agar, blood agar and chocolate agar after overnight incubation on day three, day four and finally on day seven[8]. The growth obtained was identified by conventional biochemical tests and the antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method. Zone

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diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines as used by Tiwari et al[8]. Bacterial sensitivity was tested for the following antimicrobials: amikacin, amoxicillin clavulanic acid, ampicillin, aztreonam, cefotaxime, ceftazidime, ceftriaxone, cephalexin, cefoxitin, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin, linezolid and vancomycin.

Methicillin resistance in *Staphylococcus aureus* (MRSA) was tested using Mueller-Hinton agar with 4% NaCl with cefoxitin disc (30 micrograms) by Kirby-Bauer disc diffusion method. A zone size of >22 mm was considered sensitive and < 21 was considered as resistant. Suspected extended-spectrum beta lactamases (ESBLs) producing organisms were confirmed by double disk synergy test[8]. Detection of plasmid-mediated AmpC was done by the AmpC disk test and the isolates showing reduced susceptibility to carbapenems (imipenem and meropenem) were selected for detection of metallo-beta lactamases (MBLs) enzymes by imipenem-EDTA disk method. For quality control of disc diffusion tests ATCC control strains of *E. coli* ATCC 25922, S. *aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 strains were used. The results were expressed as percentages. Microsoft excel was used for the interpretation of these results.

III. Results

Table-1 presents the percentage of children of Bacteremia according to demographic profile. The overall incidence of bacteremia was 23.1%. The incidence of bacteremia was higher among the chidren of age group 13-60 month (38.1%) than <1 month (23.4%) and 1-12 month (12.5%). The male (25.8%) children were affected than females (19.6%).

The *Klebsiella species* organism was the most common organism which was 44.4% followed by *Staphylococcus. Aureus* (14.8%) and *Coagulase Negative staphylococci (CONS)* (11.1%). The percentage of other organism was less than 10% (Fig.1).

The percentage of gram negative bacterial isolate was among 70.4% of the samples and gram negative was 29.6% (Fig.2).

Table-2 depicts antibacterial resistance pattern of the gram negative blood stream isolates. Klebsiella spp organism was resistance to ampicillin (3), amoxyclav (5), amikacin & gentamycin (2) and one each tocotrimoxazole, ciprofloxacin, cefotaxime& aztreonam. The frequency of other organism resistance to most of drugs was one.The frequency gram positive isolates resistance to most of the drugs was one (Table-3).

IV. Discussion

Who guidelines for management of acute illness in children (Integrated Management of Childhood Illness) recommend use of an appropriate antibacterial drug in addition to an antimalarial drug in children with certain signs of severe illness[9]. Despite considerable progress in hygiene, antimicrobial therapy, and supportive treatment, blood stream infections remain important causes of morbidity and mortality which, may reaches to 20%-30%[10]. Microbiologic culturing of blood is the only available means for diagnosis of these infections and allows for successful recovery of bacteria in 99% in patients with bacteremia of septicemia[11]. An American review covering a 50-years period has show major changes in the etiology of neonatal septicemia[12].

In this study, the incidence of bacteremia was higher among male children. Similar findings had been reported in southern state of India[8]. Some other studies had also reported higher incidence of bacteremia in male children[13, 2]. The incidence of bacteremia was higher among 13-60 month (38.1%) in this study which was contradictory to the studies by Tsering et al[14] and Meremkwer et al.[2] in which incidence of bacteremia was more common among newborns. In this study, the culture positivity rate was found to be 23.1% (23/117). Almost similar rate had been reported in a Indian study in which blood culture positivity rate of 25%[8]. Other studies had also reported similar positivity rate[15, 14].

The percentage of gram negative organism found in this study was similar to other studies also[16,15]. In the present study, the *Klebsiella* species was the commonest isolate associated with bacteremia which was similar to study by Al-Charrakh et al[17]. Many studies have been shown that Gram positive organisms were the mainly *Staphylococcus aureus* as the most frequently isolated bacteria causing bacteremia[18,19].

There was varying number of the gram negative and positive organisms resistance to different drugs in this study. Prabhu et al[20] reported that the gram negative organisms showed maximum resistance to ampicillin. However, Tiwari et al[8] reported that the gram positive organisms showed 77.78% resistance to penicillin but were 100% sensitive to linezolid and vancomycin. Among the 6 *Staphylococcus aureus*, 2(33.33%) were detected as Methicillin resistant *Staphylococcus aureus* (MRSA).

One of the limitation of this study is lesser sample size. Studies on larger sample size is recommended for better interpretation of the results. The studies on the community acquired blood stream infections is also needed at present.

V. Conclusion

The resistance of the recovered *Klebsiella* spp. isolates to a number of antimicrobial agents was determined, and a pattern of multiresistance was observed which may explain the prevalence of these isolates in pediatric bacteremia.

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References Références Referencias

1. Campbell A.G.M. and Mclutosh N., Forfar and Arnil's text book of pediatrics, 5th Ed. ELBS Churchill Livingston Educational, low priced books, Scheme funded by British government, 1341-1343 (1998).
2. Meremkwer MM, Nwachukwu CE, Asuquo AE, Okebe J, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. BMC Infect Dis. 2005; 5:110-5.
3. Nwadioha SI, Nwokedi EOP, Kashibu E, Odimayo MS, Okwori EE. A review of bacterial isolates in blood cultures of children with septicaemia in a Nigerian tertiary Hospital. Afr J Microbiol Res. 2010;4:222-5.
4. Bahwere P, Levy J, Hennart P, et al. Community-acquired bacteremia among hospitalized children in rural central Africa. Int J Infect Dis. 2001; 5:180–88.
5. Idoko JA, Lawande RV, Mohammed I. A prospective study of septicaemia in Zaria, northern Nigeria. East Afr Med J. 1986; 63:515–21.
6. Al-Waznee W.S., Study on septicemia in immunocompromised patients, M.Sc. thesis, College of Science, University of Babylon, (2001).
7. Torpy J.M., Lynm C. and Glass R.M., Fever in infants, JAMA, 2004; 291, 1284-1286.
8. Tiwari Devendra Kumar, Golia Saroj, Sangeetha K.T., Vasudha C.L. A Study on the Bacteriological Profile and Antibiogram of Bacteremia in Children Below 10 Years in a Tertiary Care Hospital in Bangalore, India. Journal of Clinical and Diagnostic Research. 2013 Dec, Vol-7(12): 2732-2735.
9. Who, Unicef. Integrated management of childhood illness chart booklet—standard. Geneva: World Health Organization; 2008.
10. Washington, J.A. and Iistrup, D.M.. Blood cultures issues and controversies. Rev. Infect. Dis., 1986; 8: 792-802.
11. Wagner, G.E.. Bacteremia and septicemia. In: kingsbury, D.T., and Wagner. G.E. (eds.) Microbiology. 2nd edition. John Wiely & Sons. Inc., USA. pp. 315-320; 1990.
12. Fredman, R.M., Ingram, D.L., Gross, I., Ehrenkranz, R.A., Warshaw, J.B., and Baltimore, R.S., A half century of neonatal sepsis at Yale, 1929 to 1978. Am. J. Dis. Child., 1981; 135: 140-144.
13. Baltimore RS, Bogue CW. Infections Disease - Foci of Infection. In: Burg FD, Polin PA, Ingelfinger JR, Gershon AA, Editors. Gellis & Karan's current pediatric therapy. 17th Edition. USA: Elsevier Science; 2002; 39-256.
14. Tsering D C, Chanchal L, Pal R, Kar S. Bacteriological Profile of Septicemia and the Risk Factors in Neonates and Infants in Sikkim. J Glob Infect Dis., 2011 Jan- Mar; 3(1): 42-45.
15. Sharma M., A. Yadav, N. Goel, U. Chaudary. Microbial profile of septicemia in children. Ind Jr for the practicing doctor. Vol 5, No.4 (2008-09-2008-10).
16. Ali Z. Neonatal bacterial septicaemia at the Mount Hope Women's Hospital, Trinidad. Ann Trop Paediatr. 2004; 24(1):41-4.
17. Al-Charrakh Alaa H, Al-Muhana, Al-Saadi Zainab. Bacterial Profile of Blood Stream Infections In Children Less Than Three Years Old. J. Babylon Univ. 2005.10(30): 481-85.
18. Koneman EW, Allen SD, Janda WM, Schreckember PC, Winn WC. Koneman's Colour Atlas and text book of Diagnostic Microbiology. 6th edition. Newyork: Lippincott; 2006; 97-99.
19. Mohammad A. Bacteremia among Jordanian children at Princess Rahmah Hospital: Pathogens and antimicrobial susceptibility patterns. Iran J Microbiol. 2010; 2(1): 22-26.
20. Prabhu K, Bhat S, Rao S. Bacteriologic profile and antibiogram of blood culture isolates in a pediatric care unit. J Lab Physicians. 2010; 2:85-8.

Table 1 : Percentage of children of Bacteremia according to demographic profile

	No. of children assessed	Bacteremia	
		No.	%
Age group			
<1 month	64	15	23.4
1-12 months	32	4	12.5
13-60 month	21	8	38.1
Gender			
Male	66	17	25.8
Female	51	10	19.6
Total	**117**	**27**	**23.1**

Table 2 : Antibacterial resistance pattern of the gram negative blood stream isolates

Antibiotics	Klebsiella spp (n=12)		E.coli (n=2)		Pseudomonas aeruginosa (n=2)		Acinetobacter baumanii (n=1)		S.typhi (n=2)		Citrobacter freundii (n=1)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	3	25.0	1	50.0	NT	-	NT	-	2	100.0	1	100.0
Amoxyclav	5	41.7	0	0.0	NT	-	NT	-	1	50.0	0	0.0

Amikacin	2	16.7	1	50.0	1	50.0	0	0.0	0	0.0	1	100.0
Cotrimoxazole	1	8.3	1	50.0	NT	-	0	0.0	1	50.0	0	0.0
Gentamycin	2	16.7	0	0.0	1	50.0	1	100.0	0	0.0	0	0.0
Tobramycin	NT	-	NT	-	1	50.0	0	0.0	NT	-	NT	-
Ciprofloxacin	1	8.3	0	0.0	2	100.0	1	100.0	1	50.0	1	100.0
Cefotaxime	1	8.3	1	50.0	NT	-	NT	-	1	50.0	0	0.0
Ceftriaxone	2	16.7	0	0.0	NT	-	NT	-	0	0.0	0	0.0
Ceftazidime	NT	-	NT	-	0	0.0	1	100.0	NT	-	NT	-
Aztreonam	1	8.3	0	0.0	0	0.0	0	0.0	NT	-	0	0.0
Imipenem	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

NT-Not Tested

Table 3 : Antibacterial resistance pattern of gram positive blood stream isolates

Antibiotics	Staphylococcus aureus (n=4)		CONS (n=3)	
	No.	%	No.	%
Penicillin	2	50.0	1	33.3
Amoxyclav	1	25.0	1	33.3
Cefoxitin	1	25.0	2	66.7
Erythromycin	2	50.0	1	33.3
Linezolid	1	25.0	0	0.0
Vancomycin	0	0.0	1	33.3

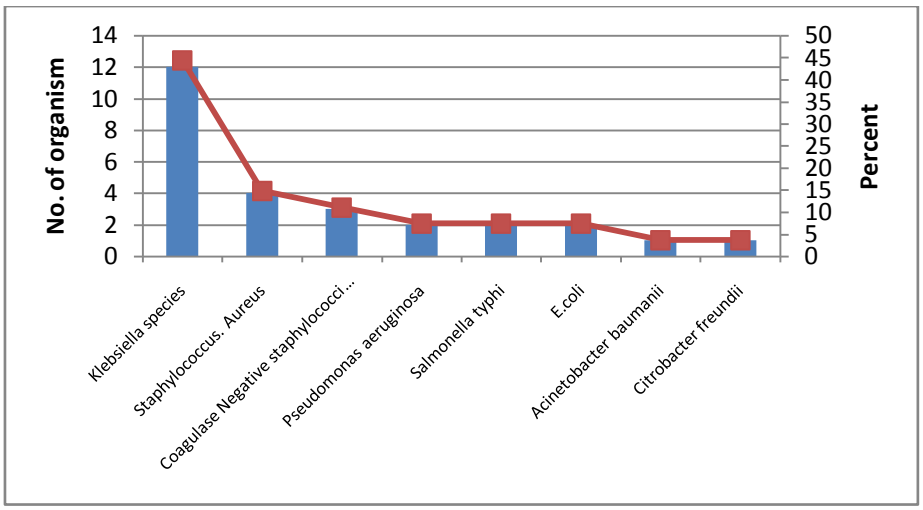

Figure 1 : Distribution of organisms isolated from blood culture

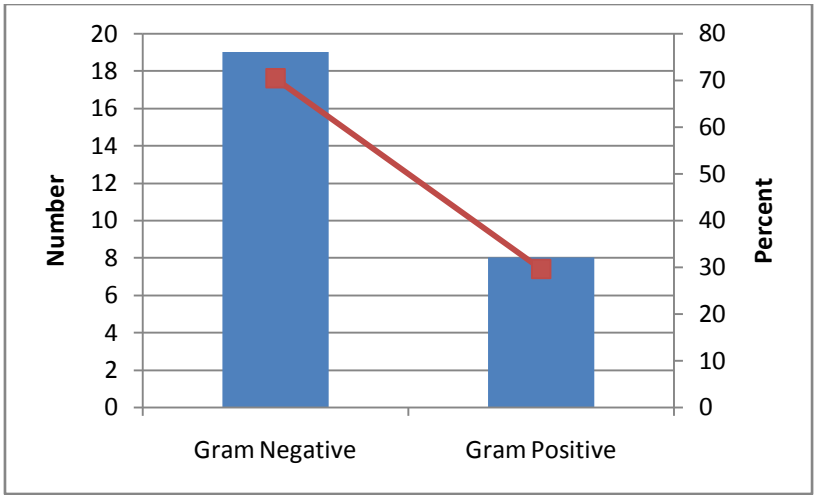

Figure 2 : Prevalence of gram negative and gram positive bacterial isolates

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A Study on Cold Agglutinins in Malaria from a Tertiary Care Hospital of South India

By Venkatesh V. N, Ravish Kumar M & Nagendra Gowda M. R
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Abstract- *Background:* Malarial infection associated with common hematological abnormalities like anemia, Thrombocytopenia, Hemolytic anemia. Hemolytic anemia is due to occurrence of cold agglutinins. This study throws light on the prevalence of cold agglutinins in patients with malarial infection and their detection.

Method: About 150 patients diagnosed as having Malaria by Peripheral smear, QBC for malaria or by Rappid diagnostic test for Malaria were studied for occurrence of cold agglutinins by Cold agglutinin test.

Results: Out of 150 Patients, who are diagnosed as positive for malaria, 83.3% of the patients had negative for cold agglutinin and only (4%) were found to have high titers of cold agglutinins. The mean age group of the patients who were positive for the test was 45.25 ± 19.7 years. The Hemoglobin percentage and platelet count in cold agglutinin positive patients were 8.85 (± 3.72) gms% and 1, 03, 500 (± 1.16) respectively.

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Conclusion: The prevalence of cold agglutinins in patients infected with Plasmodium vivax or P falciparum was 2.67%. There was a significant change in hematological parameters like hemoglobin but not platelets.

I. Introduction

Malaria is a public health problem in Sub Saharan Africa, some parts of South East Asia and South America with considerable morbidity and mortality. This disease was almost eradicated in India during 1960's but to reemerge as a public health problem in last few decades[1]. This is mainly due to development of resistance by mosquitoes against the insecticides and to therapeutic agents by the plasmodium organism. The social and environmental changes also plays major role in this situation. Usually the disease malaria results from the parasites belonging to Plasmodium species. Human malaria is a result of four different species of plasmodium including Vivax, Falciparum, Ovale and Malariae. Nowadays, Plasmodium Knowlesii a fifth parasite which was known cause malaria in monkeys has been implicated in some parts of Karnataka.

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Simple techniques including examination of the peripheral smear is used to establish the diagnosis in Malaria. Fluorescent technique can also be employed to detect the malarial parasite. Nowadays, Malaria is often considered as grey area between the parasitology and hematology. Recent text books have considered malaria as a typical example of Hemolytic Anemia as result of acquired extra corpuscular causes. The common hematological abnormalities accompanied with the malarial infection are anemia, thrombocytopenia, spleenomegaly, mild to moderate atypical lymphocytosis and rarely DIC.[2]

The available literature had shown that, the main reason for hemolytic anemia especially in malaria is due to occurrence of cold agglutinins. The cold agglutinins are capable of agglutinating RBCs and hence result in hemolysis. But the literature available shows that the cold agglutinins occur transiently in malaria. This is mainly due to activation of polyclonal B lymphocytes which is predominantly of IgM variety. Rarely might they be either IgA or IgG variety which has the specificity for blood group antigen. The literature suggests that the hemolysis appears in 2 – 3 weeks after the malarial infection. The hemolysis is usually mild and self limiting and occasionally severe and fatal.[3, 4]

Increasing emphases is now given for these antibodies in protection against human malaria which is directed at erythrocytic stages of Plasmodium falciparum. But the protection offered by such antibodies is relatively unstable and the precise role of specificities remains unclear regarding the antigenic variability of parasite proteins. Even though parasite specific antibodies formed contribute to protection, it is not evident to what extent antibodies so formed contribute to protection[5]. Many studies have shown that these antibodies result in hemolytic anemia due to complement mediated RBC destruction in the reticuloendothelial system.

Studies regarding role of cold agglutinins are scant in India and World. This made us to take up this study in order study the profile of patients with Cold agglutinins in Malaria.

II. Materials and Methods

This cross sectional study was undertaken in the Department of Microbiology, Basaveshwara Medical College and Hospital, Chitradurga. About 150 patients who were diagnosed as having Malaria by peripheral

smear examination (both thick and thin), MPQBC or by malarial antigen assay before starting the antimalarial treatment were included in the study. An informed consent was obtained from each patient before the study was started and clearance from Intuitional ethical committee was obtained. This study was carried out between January, 2012 to March, 2013.

A detailed history was taken followed by detailed clinical examination for all the patients included in the study. These patients were also investigated for Hemoglobin estimation by cyanmethemoglobin method and Total platelet count by modified Dacie Leurs method.

Cold agglutinin test was used to detect cold agglutinins in the malarial patients. The cold agglutinins if present in patient's serum in high titers may be pathologic and result in cold agglutinin disease. Serum or plasma of all the malarial patients was separated at 37^0 C from a Blood sample collected. A pool of 2 or more examples of washed group O1 adult red cells and Phosphate buffered saline (PBS) at pH 7.3 were used as reagents. A serial two fold dilutions of the patient's serum or plasma in PBS were prepared followed by Two drops of each dilution 1 drop of a 3% to 5% suspension of red cells was mixed. The solution was mixed and incubated at 4^0 C for 1 to 2 hours.

The tubes were centrifuged for 15 to 20 seconds at 900 to 1000 X g. Then it was placed in ice water bath. The tubes were examined microscopically for agglutination, starting with the tube at highest dilution. The results were graded and recorded.

All the details were collected in a pre-structured, self administered proforma. The data thus obtained was compiled and analysed in the form of frequency and proportions. Chi square test and Student T test were used as significance test by using Statistical Package for Social Sciences (SPSS vs 18). A p value of less than 0.05 was considered as statistically significant.

III. Results

Table 1 : Cold agglutinin test results of the study group

	Frequency	Percent
Negative	125	83.3
Positive at 4 deg at 1/256 dilution	3	2.0
Positive at 4 deg at 1/64 dilution	1	0.7
Positive at 4 deg at 1/32 dilution	3	2.0
Positive at 4 deg C at 1/16 dilution	5	3.3
Positive at 4 deg at 1/8 dilution	10	6.7
Positive at 4 deg at 1/4 dilution	3	2.0
Total	150	100.0

On observing the results cold agglutinin test, about 83.3% of the patients had negative cold agglutinin test. Only 4 patients who had malaria were positive for the cold agglutinin test since the cut of value for the positive test was above 1:64 dilution.

Table 2 : Distribution of the Malarial patients according to age group and result of Cold agglutinin test

Age group	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Less than 20 yrs	25 (17.1)	1 (25.0)	26 (17.3)
21 - 30 yrs	57 (39.0)	0	57 (38.0)
31 - 40 yrs	25 (17.1)	0	25 (16.7)
41 - 50 yrs	17 (11.6)	2 (50.0)	19 (12.7)
51 - 60 yrs	10 (6.8)	0	10 (6.7)
61 yrs and above	12 (8.2)	1 (25.0)	13 (8.7)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	34.09 ± 15.55	45.25 ± 19.7	34.39 ± 15.7

t (148)= 1.406 p=0.162, NS

Table no 2 shows the age group wise distribution of the study group. The mean (± SD) age of subjects in the study group was 34.39 ± 15.7 years. The mean age group of the patients who were positive for the test was 45.25 ± 19.7 years. About 50% of the subjects who had positive test belonged to 51 – 60 years age group, one patient was aged less than 20 years and another aged more than 60 years. There was no statistically significant difference between the age of the patients and result of the Cold agglutinin test.

Table 3 : Distribution of the Malarial patients according to sex and result of Cold agglutinin test.

Sex	Cold agglutinin test		Total
	Negative n (%)	Positive n (%)	n (%)
Male	104 (71.2)	3 (75.0)	107 (71.3)
Female	42 (28.8)	1 (25.0)	43 (28.7)
Total	146 (100)	4 (100)	150 (100)

χ^2 value = 0.027 df=1 p=0.869

About 75.0% of the patients who were positive for the cold agglutinin test were males and 25% were females. Of 146 patients who had shown negative results for the test, 71.2% were males and 28.8% were females. This difference was not statistically significant.

Table 4 : Distribution of the Malarial patients according to the level of hemoglobin and result of Cold agglutinin test.

Haemoglobin %	Cold agglutinin test		Total
	Negative n (%)	Positive n (%)	
More than normal	35 (24.0)	2 (50.0)	37 (24.7)
Less than normal	111 (76.0)	2 (50.0)	113 (75.3)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	11.40 ± 2.67	8.85 ± 3.72	11.33 ± 2.72

t (148)= 1.862 p=0.065, NS

The mean hemoglobin level of the study subjects was 11.33 (± 2.72) gm%. Patients negative cold agglutinin test results had a mean hemoglobin level of 11.4 (± 2.67) gm% and those with positive test had a mean hemoglobin level of 8.85 (± 3.72) gm%. About 50% of the patients had normal level of hemoglobin and 50% had less than normal level of hemoglobin. There was statistically significant difference in the hemoglobin levels between the negative and positive cold agglutinin test patients.

Table 5 : Distribution of the malarial patients according to the level of Platelets and result of Cold agglutinin test

Platelet Count	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Less than 1,65,000	132 (90.4)	3 (75.0)	135 (90.0)
Normal	13 (8.9)	1 (25.0)	14 (9.3)
More than 4,15,000	1 (0.7)	0	1 (0.7)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	97,832.2 ± 63,824	1,03,000 ± 1.16	97,970.0 ± 65,099.4

t (148)= 0.156 p= 0.876, NS

The mean platelet count in test negative patients was 97,832.2 (± 63,824) and in test positive patients was 1, 03,500 (± 1.16). This difference was not statistically significant. The platelet count was low in 91.4% of test negative and 75% of the test positive patients.

Table 6 : Distribution of the Malarial patients according to the type of malarial parasite and result of Cold agglutinin test

Type of Malarial Parasite	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Pl. Vivax	98 (67.1)	0	98 (65.3)
Pl. Falciparum	32 (21.9)	2 (50.0)	34 (22.7)
Mixed	16 (11.0)	2 (50.0)	18 (12.0)
Total	146 (100)	4 (100)	150 (100)

$\chi^2 = 8.985$ df=2 p=0.011, Sig

Table no 6 shows the type of malarial parasitic infection and results of the Cold agglutinin test. About 67.1% of the test negative patients were infected with Plasmodium Vivax. About 50% of the malarial patients with positive cold agglutinin test were infected with Plasmodium vivax and 50% had mixed Vivax and Falciparum infection. The difference in type of parasite and results of the Cold agglutinin test was statistically significant.

IV. Discussion

This study was mainly undertaken to study the prevalence of cold agglutinins in malaria patients. The results from this study had shown that about 2.67% of the malarial patients were positive for cold agglutinins with titres above 1:64 dilutions. In a study in 1980 the authors have failed to demonstrate any cold agglutinins in 1980.[6] A study of monoclonal gammopathies of cold agglutinin disease in mayo clinic had shown that the prevalence was 1.1%. A study by Torres et al had shown prevalence similar to that observed in this study.[7]

The mean age group of the patients who were positive for the test was 45.2 (± 19.7) years. About 50% of the patients who were positive for the test belonged to 41 – 50 years and 61 years and above age group. However there was a statistically significant difference between age group and result of the Cold agglutinin test. The finding from our study shows that the prevalence of cold agglutinins was common in the patients above 40 years. In contrary to these findings, Sharon[4] states that the secondary cold agglutinin disease is common in children and young adults. About 75% of the patients who had cold agglutinins were males in this study.

The mean haemoglobin levels in the positive patients for cold agglutinins was 8.85 (± 3.72) gm% in this study. About 50% of the patients had hemoglobin more than normal and 50% had hemoglobin below normal. This study is able to demonstrate haemolysis similar to the study by Gertz et al[18]. There was no drop in platelet count in patients who were positive for cold agglutinins in this study. Reduced levels of Haemoglobin and Platelet count in malaria patients were recorded by other author [8].

About 67.1% of the test negative patients were infected with Plasmodium Vivax. About 50% of the patients' positive for Cold agglutinin test had mixed malarial parasitic infections. The difference in type of parasite and results of the Cold agglutinin test was significant. A study from Ouagadougou also reported the similar results.

References Références Referencias

1. Murthy GL, Sahay RK, Srinivasan VR, Upadhaya AC, Shantaram V, Gayatri K. Clinical profile of falciparum Malaria in a tertiary care hospital. J Indian Med Assoc 2000; 98(4): 160-2, 169.
2. Layla A.M. Bashawri, Ahmed A M, Ahmed AB, Mirghani AA, Malaria: haematological aspects, Annals of Saudi Medicine, Vol 22, Nos 5-6, 2002. pp 372 – 77.
3. Claudio DR, Pierre D, Loic M, Jean CH, Marc G. Specificity of auto antibodies in malaria and the role of polyclonal activation, Transaction of The Royal Society of Tropical Medicine and Hygiene 1983; 77; 2:185-8.
4. Sharon GG, Richard FL, Rajalakshmi M, Harry L M. eMedicine Cold Agglutinin Disease 2002 October 23;vol 3(10) : [5screens] http://emedicine. meds cape.com/article/135327 treatment. Accessed on August 28, 2009.
5. Constantin F, Luis F G, Adolfo SN, Alysson C, Cor Jesus F F, Erika M B, Nelson M V, Increased polyclonal immunoglobulin reactivity toward human and bacterial proteins is associated with clinical protection in human Plasmodium infection, Malaria Journal 2005, 4:5.
6. Facer CA, Direct antiglobulin reactions in Gambian children with P. falciparum malaria, Cltn. Exp. Immunol. 1980, 39, 279-288.
7. Torres JR, Villegas L, Perez H, Suarez L,Torres VMA, Campos M. Low grade parasitaemia and cold agglutinins in patients with hyper-reactive malarious splenomegaly and acute haemolysis. Ann Trop Med Parasitol 2003; 97(2): 125–30.

8. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L et allmpact of plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malaria Journal 2010;9 (suppl 3):S4 2-11.

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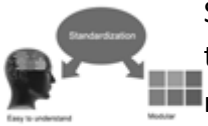

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Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

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

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

The Institute will be entitled to following benefits:

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

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

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

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

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

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

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

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

The following entitlements are applicable to individual Fellows:

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

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

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

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

Other:

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

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

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

Note :

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

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Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

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Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

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

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

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

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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

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

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

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Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. 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.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final Points:

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

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

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

General style:

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

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

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

In every sections of your document

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

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

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

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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

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

Title Page:

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

Abstract:

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

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

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

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

Approach:

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

Introduction:

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

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

Approach:

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

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

Procedures (Methods and Materials):

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

Materials:

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

Methods:

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

Approach:

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

What to keep away from

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

Results:

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

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

Content

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

What to stay away from

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

Approach

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

Figures and tables

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

Discussion:

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

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

Approach:

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

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

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

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

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

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

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

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

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 Inc. (US).

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

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save our planet

Global Journal of Medical Research

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