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List of Publications

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2. Kumar CA, Gaikwad D, Baveja S. Clinical and haematological profile in malaria at a tertiary care hospital. *J. Evolution Med. Dent. Sci*.2018; 5 (66): 4692-4695.

3. Gaikwad D, Dengale P. Improving hand hygiene compliance. *IOSR J Nur Health Sci.*2020;9(6): 01-06.

4. Gaikwad D, Sonawane A, Baveja S. Fatal empyema thoracis due to Nocardia asteroides in a neonate. *IOSR J Den Med Sci*. 2020;19(11):50-52.

5. Gaikwad D, Kumar CA, Baveja S. Comparative Staining Methods for Microscopic Diagnosis of Malaria. *Paripex Indian J. Res.* 2016; 5(8):236-237.

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STUDY OF THREE MALARIA RAPID DETECTION TESTS AND ITS CORRELATION WITH PARASITIC INDEX FOR P.FALCIPARUM AND P.VIVAX

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ABSTRACT **OBJECTIVE:** To determine the sensitivity and specificity of three Malaria rapid antigen detection tests(RDTs) .To study the sensitivity of the RDTs in relation to parasitic index. **MATERIALS AND METHODS:** The study was conducted at a tertiary care hospital. Peripheral smear were prepared and stained.Parasite index was calculated. Three rapid antigen detection tests ; Optimal – IT, Paramax-3 and QDx malaria PAN/PF were tested. **RESULTS:** Sensitivity for Optimal – IT was highest (98.47%). QDx malaria PAN/Pf gave highest specificity (97%). All the three RDTs gave sensitivity of 100% at a parasitic index of more than 100 parasites per µl. Sensitivity of Optimal – IT and QDx malaria PAN/Pf for P falciparum and P vivax was 100% and 88.88% respectively at a parasitic index of 51-100 parasites/ µl. **CONCLUSION:** Malaria RDTs are a good diagnostic tool in health care set ups where quick results are desired and expert microscopy is not available.

KEYWORDS : Malaria, Rapid Antigen Detection Tests, Optimal – IT, Malaria Diagnosis, Malaria Parasitic Index

INTRODUCTION

Malaria has been known since ancient times and has been diagnosed based on patient's signs and symptoms. Today with increasing chloroquine resistance and widespread use of expensive arteminisin combination therapies, an accurate and rapid diagnosis is essential. Clinical features of malaria have been described from Hippocratus to Osler.[1] In 1891, Romanowky introduced staining methods for these parasites.[2,3] Today more than a century later, microscopic detection and identification of Plasmodium species in Giemsa stained blood films remains the gold standard for laboratory diagnosis. Methods using advances in technology have been introduced as alternatives to light microscopy.[4] Becton and Dickenson developed a fluorescent staining technique using a capillary tube called as Quantitative Buffy Coat test in 1991-92.[2] Polymerase chain reaction (PCR) based methods have been used since early 1900's for the detection of Plasmodium parasites in human patients.[5] Many non-microscopic, rapid dip stick tests based on the detection of various antigens of malaria parasites were introduced in the early 1990s.[6] P.falciparum Histidine Rich Protein II (1987), parasite aldolase (1992) and parasite Lactate dehydrogenase (1998) are the target antigens used for such tests.[2]

Several commercial kits are available detecting different malaria antigens like the Histidine rich protein 2 (HRP-2), parasite lactate dehydrogenase (pLDH) and aldolase. These tests can be performed in 15-20 minutes. Reporting the test results of the Rapid detection tests (RDT's) also does not require the expertise as it is for microscopy. There are several Rapid antigen detection tests available. The choice of an appropriate assay would help in accurate and rapid malaria diagnosis.

MATERIALAND METHODS

The present study was carried out at a tertiary care hospital after approval by the Ethical committee of the parent college.

Two hundred blood samples were obtained from patients of all ages with clinical suspicion of malaria. Patients who came for follow-up visits of an earlier episode of malaria or within 4 weeks post treatment were excluded from the study. Patients with history of treatment with antimalarials were excluded from the study.

From each case 2 ml. of blood was collected into an ethylenediamine tetra acetic acid bulb(EDTA) and one thick and thin blood film each was prepared on different slides and stained with Giemsa stain as per standard protocol. A thick smear was considered negative if no parasites were seen in at least200 fields.[7]

For examination of parasite density Giemsa stained thick blood films were scored by counting the total number of asexual parasites per 200 white blood cells (WBCs) and the parasite index was calculated using the following formula.[8]

$$\frac{\text{Parasites counted} \times 8000}{200} = \text{parasite count per microlitre of blood}$$

Thin films were examined for at least 100 fields in not more than 10 minutes.[3] Speciation of the parasites was done by examining stained thin films. Three malaria rapid antigen detection kits (RDTs) were used and compared with peripheral smear. The three RDTs were :

Optimal - IT (DiaMed, Switzerland), Paramax -3 (Zephyr Biomedicals, India), and QDx MALARIA PAN / Pf (Piramal Healthcare, India).

Optimal - IT (DiaMed, Switzerland)

DiaMed Optimal – It is an immunochromatogrphic test, using monoclonal antibodies against the metabolic enzyme pLDH of Plasmodium spp. These monoclonal antibodies are classified in two groups; one specific for Plamodium falciparum and the other is a pan - specific monoclonal antibody which reacts with all four species of Plasmodium spp. which can occur in human beings: P.falciparum, P.vivax, P.ovale, P.malariae

Paramax -3 (Zephyr Biomedicals, India)

It is a rapid, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of P.falciparum specific histidine rich protein-2 (Pf. HRP-2), P.vivax specific pLDH and pan malaria specific pLDH.

QDx MALARIA PAN / Pf (Piramal Healthcare, India)

QDx Malaria PAN / Pf is a qualitative, sandwich immunoassay for the detection and differentiation of P.falciparum specific histidine rich protein-2 (Pf. HRP-2) and pan malaria specific pLDH.

Rapid diagnostic tests were read by the same bacteriologist and confirmed by a second independent reader when needed, all according to the manufacturer's instructions.

Statistical analysis: The performance of RDTs was expressed by calculating the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV), for P. vivax and P. falciparum separately, taking microscopy results as the "gold standard". The sensitivity and specificity of the gold standard was taken as 95%. The sample size of the study was 200. Based on this, standard error was calculated to be 1.54. The normal range of sensitivity and specificity is 95% 1.54% ie 93.46%-96.54%. Data were analyzed in SPSS. Proportions were compared using the Chi – square

test.

RESULTS

Of the 200 samples processed in the laboratory, 132 had positive blood films. Ninety four of the 132 infecting species were identified as P.vivax, 33 as P.falciparum, and 5 as mixed infections.

To assess the performance of optimal-IT and QDx MALARIA PAN / Pf for diagnosis of P.vivax, cases with mixed infections with P.falciparum were excluded, because the pan –malaria antigen turns up positive due to P.falciparum infection. For performance of P.falciparum detection, mixed infections with P.falciparum were included. One slide which had only gametocytes was not included in further analysis.

TABLE 1

Based on the above observations OptiMAL-IT had a sensitivity and specificity of 98.47% and 95.58% respectively. The Positive predictive value (PPV) and Negative predictive value (NPV) was 97.72% and 97.01% respectively.

Sensitivity and specificity obtained for Paramax-3 was 95.41 %and 94.11% respectively. It had a PPV of 96.89% and NPV of 91.42 %.

The sensitivity and specificity recorded for QDxMALARIA PAN/Pf was 96.94% and 97.05% respectively. The PPV and NPV was 98.44% and 94.28% respectively.

The confidence interval calculated for the sensitivity and specificity of Giemsa stained peripheral blood smear which was the gold standard was found to be 93.46% - 96.54%. The sensitivity and specificity obtained for QDxMALARIA PAN/Pf was higher than this confidence interval, thus giving better results than microscopy. The sensitivity for OptiMAL-IT was also higher than the normal range for microscopy. The specificity for OptiMAL-IT and sensitivity and specificity for Paramax-3 is within the confidence interval giving comparable results with microscopy.

TABLE 2

Sensitivity of OptiMAL-IT for P.falciparum was 97.29%. The specificity recorded was 99.38%. The PPV and NPV obtained was 97.29 % and 99.38% respectively.

Based on the above observations Paramax-3 had a sensitivity and specificity of 94.59% and 98.76 % respectively for P.falciparum. The PPV and NPV was 94.59 % and 98.76 % respectively.

QDxMALARIA PAN/Pf recorded a sensitivity and specificity of 97.29 % and 99.38% respectively when tested for P.falciparum. The PPV observed was 97.29% while the NPV was 99.38%.

The sensitivity and specificity for OptiMAL-IT and QDxMALARIA PAN/Pf for P.falciparum was higher than the upper limit of confidence interval (93.46%- 96.54%). The specificity for Paramax-3 was also high while its sensitivity was within the range. Thus rapid tests gave either better or comparable results for P.falciparum when compared with microscopy.

TABLE 3

Sensitivity of OptiMAL-IT for P.vivax was 98.93%. The specificity recorded was 98.00%. The PPV and NPV obtained was 97.89 % and 98.98% respectively.

Paramax-3 had a sensitivity and specificity of 95.95% and 98.00 % respectively for P.vivax. The PPV and NPV was 97.93% and 96.07% respectively.

QDxMALARIA PAN/Pf recorded a sensitivity and specificity of 96.80 % and 99.00% respectively when tested for P.vivax. The PPV observed was 98.91% while the NPV was 97.05%.

The sensitivity and specificity for OptiMAL-IT and QDxMALARIA PAN/Pf for P.vivax was higher than the upper limit of confidence interval (93.46%- 96.54%). The specificity for Paramax-3 was also high while its sensitivity was within the range. Thus OptiMAL-IT and QDxMALARIA PAN/Pf for P.vivax gave better results when compared with microscopy. Paramax-3 also gave comparable results with microscopy.

TABLE 4

Percentage sensitivity for rapid tests with relation to parasitic index was lower at an index of 0-50 parasites / µl for P.falciparum cases. Percentage sensitivity for Paramax-3 at parasitic index 50-100 parasites / µl was lower than other rapid tests. Overall percentage sensitivity was higher for OptiMAL-IT and QDxMALARIA PAN/Pf as compared to Paramax-3 for P.falciparum cases.

TABLE 5

The overall percentage sensitivity with relation to parasitic index was highest for OptiMAL-IT for P.vivax cases. OptiMAL-IT gave a sensitivity of 100% at a parasitic index of 0-50 parasites/µl but one case was found to be negative with a parasitic index of 51 -100 parasites/µl.

DISCUSSION

The aim of the study was to compare three rapid antigen detection tests with peripheral blood smear for the diagnosis of malaria. The number of cases were limited to only symptomatic indoor patients with clinical suspicion of malaria. Sub-clinical infection or normal controls were not included in the study, hence the number of samples with low parasitaemia (<100 per cumm) and gametocytes only, were few in the study.

It was found that rapid tests yielded comparable results with microscopy.

OptiMAL-IT a pLDH based assay had an overall sensitivity of 98.47% and a specificity of 95.58 %. The positive predictive value (PPV) was 97.72% and negative predictive value (NPV) was 97.01%. It had a sensitivity of 97.29 % and specificity of 99.38 % for P.falciparum and sensitivity of 98.93% and specificity of 98% for P.vivax. QDx MALARIA PAN / Pf gave similar results as OptiMAL-IT. It had an overall sensitivity of 96.94 % and a specificity of 97.05 %. The PPV was 98.44 % and NPV of 94.28 %. It had a sensitivity of 97.29 % and specificity of 99.38 % for P.falciparum and sensitivity of 96.80 % and specificity of 99% for P.vivax. The results obtained for Paramax -3 were slightly lower than the above two tests. It showed an overall sensitivity of 95.41 % and a specificity of 94.11 %. The PPV was 96.89 % and NPV of 91.42 %. It had a sensitivity of 94.59 % and specificity of 98.76 % for P.falciparum and sensitivity of 95.95 % and specificity of 98% for P.vivax.

The overall sensitivity and specificity obtained for QDxMALARIA PAN/Pf was higher than the confidence interval calculated for comparing rapid tests with microscopy. Thus it gave better results than microscopy. The sensitivity for OptiMAL-IT was also higher than the normal range for microscopy. The specificity for OptiMAL-IT and sensitivity and specificity for Paramax-3 was within the confidence interval giving comparable results with microscopy.

Sensitivity of rapid tests with relation to parasite index was comparable with microscopy at a parasitic index of >100 parasites/µl. However the sensitivity dropped when the parasitic index was below 100 parasites /µl.

Several authors who have tested OptiMAL-IT for malarial diagnosis from different countries have given more or less similar results. They have reported a sensitivity and specificity in the range of 80-100%.[9] Palmer et al in Honduras has reported a sensitivity of 94% and specificity of 100% for P.vivax and sensitivity of 88% and specificity of 99% for P. falciparum.[10] From hospital of tropical diseases UK, Moody et al while conducting their study in sub-Saharan Africa, Asia and South America have reported a sensitivity of 95.3% and specificity of 100% for P. falciparum and sensitivity of 96% and a specificity of 100% for Plasmodium vivax.[11] John et al obtained a sensitivity of 94% for P. falciparum and 98.2% for P.vivax in a trial in Southern India.[12] The sensitivity and specificity of the present study falls in the range given above and is comparable to the various studies. Therefore the results of this study further substantiates that OptiMAL-IT is an effective and sensitive tool in the diagnosis of malaria.

There are no published reports for Paramax-3 and QDx MALARIA PAN / Pf except for the technical support sheet by Malaria Reseach Centre (ICMR) for testing Paramax.[13] There are several studies on other HRP2 based assays like ICT P.f/P.v, NOW-Malaria-ICT, Paracheck-Pf, ParaSight-F, and Parascreen pan/pf etc. Paramax and Parascreen malaria diagnostic tests manufactured by M/s Zephyr Biomedicals, Goa were tested at Malaria Research Centre (ICMR),

Goa, India in 2004. Both tests gave a 100% sensitivity, specificity, NPV, and PPV for both P. falciparum and P. vivax.
The parasitaemia range for P. falciparum and P. vivax was 400-22,720 and 520-33600 parasites/µl of blood respectively.[13] In the present study Paramax -3 was 100 % sensitive at a parasitic index of >100 parasites/µl.

Among the various HRP2 based tests for malaria, there is a wide range of sensitivity and specificity seen. In the present study the rapid tests showed a sensitivity and specificity at the higher range. The sensitivity and specificity for Paramax -3 was slightly lower than OptiMAL-IT and QDx MALARIA PAN / Pf. However it can identify the infection caused by P.falciparum and P.vivax separately. It can also diagnose mixed infection. The identity of the Plasmodium species helps clinicians to anticipate complications and treat the patients promptly and accurately.

In spite of over hundred published RDT trial reports, comparative assessment is difficult because i) trials do not share common guidelines; ii) clinical and epidemiological characteristics of the study populations, especially parasitemia level vary; iii) reference standards are different; even among those using Giemsa microscopy, reading rules and microscopist's skill vary; and iv) products of different lots may differ in quality or be damaged by extreme temperature or humidity during transportation and storage.[9]

HRP-2 based tests commonly give P.falciparum sensitivity of > 90 % in clinical cases. For pLDH assays, results varied among studies and product lots and variable field stability of the test could not be ruled out. Sensitivity for P.falciparum is excellent (>95%) in some studies and poorer (80 %) in others. Recent studies suggest that the tests were less sensitive for non – P.falciparum than for P.falciparum. Extremely low sensitivity had been reported earlier for both HRP-2 and pLDH tests and batch specific problems were suspected. Overall RDT specificity is commonly above 85%, approaching 100% when used in some groups of returning non immune travellers.[14]

In the present study OptiMAL-IT missed two cases which were positive on microscopy. Paramax-3 missed 6 cases while QDx MALARIA PAN / Pf missed 4 cases of malaria. The reason for these false negative results could be that rapid tests are not sensitive below a parasitic index of 100 parasite/ul.[15] This fact is also reflected in the present study, as, all the cases which were missed had a parasitic index below 100 parasite/ul on the peripheral blood smear. Besides, occasional false negative results may be caused by deletion or mutation of the hrp-2 gene. Secondly, It has been suggested that anti HRP-2 antibodies in humans may explain why some tests were negative. Presence of an inhibitor in the patient's blood preventing development of control line has also been noted.[14] pLDH is produced by living parasites. It is possible that some patients might have already taken antimalarials and not disclosed it which could also account for negative results of the RDTs.[16]

The rapid tests also gave some false positive results. OptiMAL -IT gave 3 false positive results while Paramax-3 and QDx MALARIA PAN / Pf gave 4 and 2 false positive results respectively. These may be due to a number of factors. Cross reactivity with rheumatoid factor in blood generates a false positive test line, but replacement of IgG with IgM in recent products reduces this problem. Cross reactivity with heterophile antibodies may also occur.[14] HRP -2 has been shown to persist and is detectable after the clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host. Low level parasitemia seen in areas of endemic infection because of constant exposure to the malarial parasites may also result in positive results with doubtful clinical significance.[8] Furthermore, in some infections parasites may have been sequestered and would not be detected on peripheral blood smear examination.[17]

It was observed that five cases of mixed malarial infection were seen on microscopy. OptiMAL-IT and QDx MALARIA PAN / Pf reported these cases as P. falciparum due to the configuration of the test. Paramax-3 identified these cases as mixed infections with 100% sensitivity and specificity. As the management of mixed infections includes P.vivax treatment, the format used in Paramax-3 is advantageous. One case in this study showed only the gametocytes of P. falciparum in the blood smear and no ring forms were seen. The rapid tests reported it as P. falciparum infestation. Such results can lead to unnecessary medication.

The commercially available malaria rapid detection tests in kit form are a good alternative to microscopic diagnosis for malaria especially where quick results are required or in health care facilities where there is a lack of manpower and equipments as these tests do not require extensive training or equipments to perform or to interpret the results. Besides, the tests can be performed in 15 to 20 minutes.

CONCLUSIONS

Peripheral blood smear examination remains the gold standard for diagnosis of malaria while Rapid antigen detection tests are a good alternative but cannot be a substitute. Rapid antigen detection tests have maximum utility in emergency laboratories, intensive care setups and the casualty where quick results are desired. They can also be used for malaria diagnosis where facilities for expert microscopy are not available

Table 1: Results Of Malaria Rapid Detection Tests For Malaria Positive Cases (n=199)

Results of RDTs	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT results (n=199) Positive	129	3	132
Negative	2	65	67
Paramax-3 results(n=199) Positive	125	4	129
Negative	6	64	70
QDxMALARIAPAN/Pf (n=199) Positive	127	2	129
Negative	4	66	70

Table 2: Results Of Rapid Tests For P.falciparum

Results of RDTs P.falciparum (n=199)	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT P.falciparum Positive	36	1	37
Negative	1	161	162
Paramax-3 Positive	35	2	37
Negative	2	160	162
QDxMALARIAPAN/Pf Positive	36	1	37
Negative	1	161	162

Table 3: Results Of Rapid Tests For P.vivax

Results of RDTs P.vivax (n=194)	Results of Microscopy		Total
	Positive	Negative	
OptiMAL-IT Positive	93	2	95
Negative	1	98	99
Paramax-3 Positive	95	2	97
Negative	4	98	102
QDxMALARIAPAN/Pf Positive	91	1	92
Negative	3	99	102

Table 4: Percentage Sensitivity Of Rapid Tests With Relation To Parasitic Index For P.falciparum (n=32)*

P.falciparum (n=32)* Parasites/µl	Microscopy Results	OptiMAL-IT positive (% Sensitivity)	Paramax-3 positive (% Sensitivity)	QDxMALARIA PAN/Pf positive (% Sensitivity)
0-50	3	2(66.66)	2(66.66)	2(66.66)
51-100	3	3(100)	2(66.66)	3(100)
101-500	3	3(100)	3(100)	3(100)
501-5,000	7	7(100)	7(100)	7(100)
5,001-50,000	14	14(100)	14(100)	14(100)
>50,000	2	2(100)	2(100)	2(100)
Overall	32	31(96.87)	30(93.75)	31(96.87)

* False positive cases and a case with only gametocytes was not included

Table 5: Percentage Sensitivity Of Rapid Tests With Relation To Parasitic Index For P.vivax (n=94)*

P.vivax (n=94)* Parasites/µl	Giemsa positive	OptiMAL-IT positive (% Sensitivity)	Paramax-3 positive (%Sensitivity)	QDxMALARIA PAN/Pf positive (% Sensitivity)
0-50	9	9 (100.00)	7 (77.77)	7 (77.77)
51-100	9	8 (88.88)	7 (77.77)	8 (88.88)
101-500	16	16 (100)	16 (100)	16(100)
501-5,000	17	17 (100)	17 (100)	17 (100)
5,001-50,000	41	41 (100)	41 (100)	41 (100)
>50,000	2	2 (100)	2 (100)	2 (100)
Overall	94	93 (98.93)	90 (95.74)	91 (96.80)

* False positive cases were not included.

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CLINICAL AND HAEMATOLOGICAL PROFILE IN MALARIA AT A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

Malaria is a major global health problem. An accurate and rapid clinical and laboratory diagnosis of malaria becomes essential to decrease the morbidity and mortality caused by it. The present study was conducted to study the clinical characteristics and haematological and biochemistry parameters in malaria patients.

METHODS

The prospective study included 200 patients clinically suspected of malaria and carried out at a tertiary care hospital. A thorough history and clinical details of patients were taken. Blood (2 mL) was collected in EDTA bulb from each patient and peripheral smears prepared and stained with Giemsa stain. Haematological investigations, LFT, and RFT of the patients were noted from the patient's records. Data was statistically analysed.

RESULTS

Malarial parasites were detected in 132 of 200 (66%) patients in peripheral smears. Of these, 94 (71%), 33 (25%), and 5 (4%) were *P. vivax, P. falciparum*, and mixed infections respectively. Maximum number of cases was in age group 11 to 30 years. Male-to-female ratio was 4.7:1. Constitutional symptoms were seen in both peripheral smear positive and negative cases. CNS involvement was seen both in *P. falciparum* (24%) and *P. vivax* infection (4%), although significantly more in *P. falciparum* infections. Anaemia in *P. falciparum* (46%) and thrombocytopenia in *P. vivax* malaria (39%) was of statistical significance. Systemic manifestations and mortality were higher in *P. falciparum* (33%) than *P. vivax* (4%) infection.

CONCLUSION

Clinical signs and symptoms alone cannot be used to diagnose malaria. It has to be confirmed by laboratory diagnosis. Systemic complications and mortality is caused by both *P. vivax* and *P. falciparum*, but significantly more by *P. falciparum*. Haematological abnormalities like anaemia and thrombocytopenia should increase the possibility of malaria. Periodic analysis of the clinical manifestations with haematological and biochemistry parameters can reveal the changing behavioral patterns of the plasmodium species so that appropriate treatment and preventive measures can be taken.

KEYWORDS

Malaria, *P. falciparum, P. vivax*, Plasmodium Species.

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INTRODUCTION

Malaria is a protozoan disease caused by infections with the parasite of the genus Plasmodium and transmitted to man by certain species of infected female anopheles mosquito. Today, with the increased frequency of human migration, the disease has become a global health problem. Malaria has plagued mankind since ancient times and is still a significant threat to half of the world's population - 3.3 billion people living in 109 countries are at risk of contracting the disease. Estimates suggest that malaria afflicts between 350 and 500 million people every year.[1] India accounts for approximately two thirds of the confirmed cases reported in South East Asia Region. In 2008, 1.5 million confirmed cases were reported.

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The number has fallen from more than 2 million confirmed cases in 2001 to 1.5 million cases in 2008. About half of the cases confirmed are due to *P. falciparum*. Five states account for 60% cases: Orissa, Chhattisgarh, Madhya Pradesh, Jharkhand, and West Bengal. Other highly endemic states include Arunachal Pradesh, Assam, Meghalaya, and Tripura.[2]

OBJECTIVES

To study the clinical characteristics and haematological and biochemistry parameters in malaria patients diagnosed microscopically.

MATERIAL AND METHODS

The present study was carried out at a tertiary care hospital over a period of one and a half years after taking approval from the institutional ethics committee. The sample size was calculated according to the sensitivity and specificity of Giemsa staining and rapid diagnostic tests.[3,4]

Two hundred blood samples were obtained from patients of all ages with clinical suspicion of malaria. Patients who came for follow-up visits of an earlier episode of malaria or within 4 weeks post treatment were excluded from the study.

A through history and clinical details of the patients were noted. Blood (2 mL) was collected from each patient in an EDTA bulb and peripheral smear prepared and stained with Giemsa stain. A diagnosis of malaria was made and species identification done on noting of the morphology of the stages of parasite. The details of haemoglobin, total leucocyte and differential counts, liver and renal function tests were taken from the patient's record and noted.

Data were analysed in SPSS. Proportions were compared using the chi-square test.

RESULTS

A total of 200 patients, 163 males and 37 females were enrolled in the study. Twenty patients were admitted to the intensive care unit and the rest to the general wards.

Of the 200 samples processed in the laboratory, 132 had positive blood films. Ninety four of the 132 (71%) infecting species were identified as *P. vivax*, 33 (25%) as *P. falciparum*, and 5 (3.8%) as mixed infections.

Maximum number of cases was seen in the age group between 11-30 years. Males were affected more than females with male-to-female ratio of 4.73:1 (Table 1).

Rash, bleeding manifestations, cough, breathlessness, jaundice, oliguria, altered sensorium, and convulsions were included in the symptoms suggestive of complications. Fever was seen in 100% cases including negative patients. Constitutional symptoms were seen in both Plasmodium species and negative patients with no statistical significant difference. Symptoms suggestive of complications were seen more in cases with *P. falciparum* and the difference was statistically significant (Table 2).

Signs like pyrexia, tachycardia, hypotension, tachypnea, and bleeding diathesis were more common in malaria positive cases, but the difference was not statistically significant. Pallor was more common in *P. falciparum* and mixed cases and the finding was statistically significant. Icterus was more common in cases with *P. falciparum* with statistically significant difference (Table 3).

CNS manifestations were found to be higher in *P. falciparum* cases and the difference was statistically significant. All types of systemic manifestations were observed to be more in *P. falciparum* when compared to *P. vivax* and negative cases, but the findings were not statistically significant except for CNS complications (Table 4).

Anaemia was more common in cases of *P. falciparum* and mixed infections. Thrombocytopenia was more common in *P. vivax* cases and mixed infections. Both findings were statistically significant. Leucopenia was more common in *P. vivax* cases while hypoglycaemia was more common in *P. falciparum* cases. Deranged RFTs were higher in *P. falciparum* and mixed cases, but the findings were not statistically significant. Deranged LFTs were higher in negative and *P. falciparum* cases, but the findings were also not statistically significant (Table 5).

Mortality was observed to be the maximum in *P. falciparum* cases; 11 of 33 cases (33.33%) and this was statistically significant. Of the 94 cases of *P. vivax* 4 (4.25%) expired. No deaths were observed in the 5 cases of mixed cases. Of the 68 non-malaria cases as per peripheral smear positivity, 1 (1.47%) patient expired.

Age (Years)	Female	Male	Total
0-10	2	3	5
11-20	5	31	36
21-30	2	33	35
31-40	6	9	15
41-50	6	15	21
51-60	0	15	15
>60	2	3	5
Total	**23**	**109**	**132**

Table 1: Age and Sex Distribution in Malaria Positive Patients (N = 132)

Symptoms	P. falciparum (n=33) (%)	P. vivax (n=94) (%)	Mixed (n=5) (%)	Negative (n=68) (%)	Total (n=200) (%)	Chi square	P value
Fever	33 (100.00)	94 (100.00)	5 (100.00)	68 (100.00)	200 (100.00)	NA	NA
Chills and rigors	28 (84.84)	86 (91.48)	5 (100.00)	67 (98.52)	186 (93.00)	7.268	0.064
Nausea and Vomiting	17 (51.51)	46 (48.93)	3 (60.00)	34 (50.00)	100 (50.00)	0.273	0.965
Headache	12 (36.36)	36 (38.29)	2 (40.00)	29 (42.64)	79 (39.50)	0.476	0.924
Body ache	6 (18.18)	32 (34.04)	1 (20.00)	35 (51.47)	74 (37.00)	14.55	0.024
Symptoms suggestive of complication*	*22 (66.66)	40 (42.55)	2 (40.00)	22 (32.35)	114 (57.00)	10.712**	0.01**

Table 2: Clinical Symptoms in Cases of Malaria and its Correlation with Plasmodium Species

Clinical Signs	P. falciparum (n=33) (%)	P. vivax (n=94) (%)	Mixed (n=5) (%)	Negative (n=68) (%)	Total (n=200) (%)	Chi square	P value
Pyrexia	9 (27.27)	20 (21.27)	1 (20.00)	6 (8.82)	26 (13.00)	7.673	0.053
Tachycardia	9 (27.27)	12 (12.76)	1 (20.00)	9 (13.23)	31 (15.50)	4.372	0.024
Hypotension	3 (9.09)	7 (7.44)	0 (00.00)	2 (2.94)	12 (6.00)	NA	NA
Tachypnea	7 (21.21)	3 (3.19)	0 (00.00)	1 (1.47)	11 (5.50)	NA	NA
Pallor	*14 (42.42)	27 (28.72)	*4 (80.00)	15 (22.07)	60 (30.00)	10.493*	0.015*
Icterus	*17 (51.51)	17 (18.08)	1 (20.00)	13 (19.11)	48 (24.00)	16.43**	0.001**
Bleeding diathesis	5 (15.15)	9 (9.57)	0 (00.00)	3 (4.11)	17 (8.50)	3.943	0.268

Table 3: Clinical Signs in Malaria Cases and its Correlation with Plasmodium Species

Systemic Manifestations	P. falciparum (n=33) (%)	P. vivax (n=94) (%)	Mixed (n=5) (%)	Negative (n=68) (%)	Total (n=200) (%)	Chi square	P value
Respiratory System (RS)	8 (24.24)	15 (15.95)	1 (20.00)	8 (11.76)	32 (16.00)	2.635	0.451
Cardiovascular System (CVS)	3 (9.09)	3 (3.19)	0 (00.00)	0 (00.00)	6 (3.00)	NA	NA
Gastrointestinal Tract (GIT)	5 (15.15)	9 (9.57)	1 (20.00)	3 (4.41)	18 (9.00)	4.049	0.256
Spleen	9 (27.27)	20 (21.27)	0 (00.00)	10 (14.70)	39 (19.5)	3.666	0.300
Liver	9 (27.27)	19 (20.21)	1 (20.00)	10 (14.70)	39 (19.5)	2.297	0.513
Central Nervous System (CNS)	*8 (24.24)	4 (4.2)	0 (00.00)	7 (10.29)	19 (9.50)	11.924*	0.008*

Table 4: Distribution of Systemic Manifestations in Malaria Species and Negative Cases

Haematological & Biochemistry Parameters	P. falciparum (n=33) (%)	P. vivax (n=94) (%)	Mixed (n=5) (%)	Negative (n=68) (%)	Total (n=200) (%)	Chi square	P value
Anaemia (Hb <10 gm %)	*15(n=33) (45.45)	25(n=94) (26.59)	*4(n=5) (80.00)	22(n=68) (32.35)	66(n=200) (33.00)	9.067**	0.028**
Thrombocytopenia(Platelet count <1,50,000 cumm)	9(n=33) (27.27)	*37(n=94) (39.36)	*5(n=5) (100.00)	17(n=68) (25.00)	68(n=200) (34.00)	14.030**	0.003**
Leucopenia(TLC <4000 cumm)	4 (n=33) (12.12)	19(n=94) (20.21)	1 (n=5) (20.00)	13 (n=68) (19.11)	37(n=200) (18.50)	1.098	0.778
Hypoglycaemia	5 (n=33) (15.15)	9 (n=94) (9.57)	0(n=5) (0.00)	8 (n=68) (11.76)	22 (n=200) (11.00)	1.435	0.697
Deranged Liver Function Tests (LFT)	10(n=23) (43.47)	14(n=60) (23.33)	2(n=5) (40.00)	25 (n=55) (45.45)	51 (n=200) (35.66)	6.927	0.074
Deranged Kidney Function Tests (RFT)	13(n=27) (48.14)	25(n=76) (32.89)	3(n=5) (60.00)	15 (n=57) (26.31)	56 (n=200) (33.93)	5.460	0.141

Table 5: Distribution of Haematological and Biochemistry Parameters in Malaria Positive and Negative Cases

DISCUSSION

Of the 200 samples processed in the laboratory, 132 had positive blood films. Ninety four of the 132 infecting species were identified as *P. vivax*, 33 as *P. falciparum*, and 5 as mixed infections. Maximum numbers of cases were seen in the age group of 11-30 years. Males were affected more than females. Several authors have reported similar age and sex distribution.[5,6,7,8,9] Although, the study shows preponderance of males. Sex is not a direct factor in malaria, but may influence rates through occupation, the type of clothing used, or relative mobility.[10]

Symptoms like fever, headache, body ache, nausea, and vomiting were seen in both groups; one positive for and other negative for Plasmodium species on peripheral blood smear examination. There was no statistically significant difference seen. Signs like pyrexia, tachycardia, hypotension, tachypnea, and bleeding diathesis were more common in malaria positive cases, but the difference was not statistically significant. This emphasizes the need for a laboratory-based diagnosis rather than a complete dependence on clinical diagnosis. It would improve patient care, ensure accurate treatment of cases, and reduce morbidity and mortality of patients.

Symptoms suggestive of complications like rash, bleeding manifestations, cough, breathlessness, jaundice, oliguria, altered sensorium, and convulsions along with signs like pallor and icterus were significantly higher in *P. falciparum* cases. Systemic manifestations were also higher in *P. falciparum* cases. These findings are similar to those mentioned in literature.

Plasmodium vivax has a reputation as a benign infection, when compared with severe manifestations frequently observed with untreated *P. falciparum*. However, severe and fatal infections can occur with *P. vivax*. In the present study, *P. vivax* is seen to cause various systemic complications and the mortality observed with this species was 4.25%. More recently, *P. vivax* has shown to cause severe anaemia, respiratory distress, malnutrition, and possibly coma.[11]

CNS complications and mortality were significantly higher in *P. falciparum* when compared to *P. vivax*. In the present study, CNS involvement was seen in 8 out of 33 *P. falciparum* cases (24.24%) and 4 out of 94 cases (4.2%) of vivax malaria. Cerebral malaria remains the single most important cause of mortality in falciparum malaria. Bag et al have reported cerebral involvement in 70% patients of complicated malaria. There are infrequent reports of cerebral malaria associated with *P. vivax*. CNS involvement with *P. vivax* has been reported in only 45 cases in English literature since 1920; about half of these cases have occurred in children.[12] Thapa et al have reported 2 cases of *P. vivax* malaria complicated with seizures and symptoms of diffuse meningoencephalitis from Kolkata.[12]

In the present study, anaemia was significantly higher in *P. falciparum* cases. Hypoglycaemia, deranged LFTs and RFTs were also seen more with *P. falciparum* malaria, but not significantly so. These findings are traditionally seen more commonly with *P. falciparum* and mentioned in literature.

In the present study, thrombocytopenia and leucopenia were more common in *P. vivax* cases. Thrombocytopenia was significantly higher in *P. vivax* than *P. falciparum* cases.

Song et al have studied the clinical features of *P. vivax* malaria in Korea. Thrombocytopenia was a prominent finding in 75% of the cases at diagnosis, which resolved during therpy.[13] Memon et al when studying thrombocytopenia in hospitalised patients found (70%) patients had thrombocytopenia. 93% patients with thrombocytopenia

were *P. falciparum* positive and only 7% patients had vivax malaria.[14]

Various other studies have shown that thrombocytopenia is equally or even more common in *P. vivax* malaria contrary to the belief that abnormal haematological parameters is observed in *P. falciparum* malaria.[15,16,17] More cases of thrombocytopenia in vivax malaria infection may attribute to possible development of a new genotype of *P. vivax*.[18] Haematological abnormalities like anaemia and thrombocytopenia should increase the possibility of malaria especially when microscopy is negative taking into consideration the life cycle of the parasite.

CONCLUSION

Both *P. vivax* and *P. falciparum* can give rise to various systemic complications including cerebral malaria. Mortality is also caused by both the species, although *P. falciparum* continues to cause more complications and mortality compared to *P vivax.* Haematological abnormalities like anaemia and thrombocytopenia should increase the possibility of malaria. Periodic studies and analysis of the clinical manifestations with haematological and biochemistry parameters will reveal the changing behavioral patterns of the plasmodium species so that appropriate treatment and preventive measures can be taken.

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Improving hand hygiene compliance

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Abstract:
Background*: Proper hand hygiene is the single most important, simplest, and least expensive means of reducing the prevalence of Health care associated infections and the spread of antimicrobial resistance. The purpose of this project was to observe compliance of hand hygiene, study factors causing noncompliance and improvement following intervention*
Materials and Methods*: This was a prospective Hand Hygiene Project. It was a 6 month Quality improvement project (June 2018 to November 2018) designed to improve hand hygiene compliance at Vishwaraj hospital. Data on hand hygiene compliance were collected monthly from June 2018 to November 2018*
Results*: At baseline, hand hygiene compliance averaged 41.6% in our hospital. Data revealed nine different causes of hand hygiene noncompliance; we developed and implemented specific interventions targeted to causes of hand hygiene noncompliance. The improvements were associated with a increase in compliance from 41.6 % to 54.2 % (p<0.05), a level of performance that was sustained for 4 months through the end of the project period.*
Conclusion:*We targeted the most important causes of hand hygiene failure. Such a targeted approach is an effective improvement strategy.*
Key Word: Hand*hygiene,Hand hygiene barriers, QualityProject*

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I. Introduction

In the 1840s, Semmelweis demonstrated the efficacy of hand hygiene in dramatically reducing maternal deaths in hospitals from puerperal fever.[1] Ever since, the goal of achieving and sustaining high rates of compliance with hand hygiene protocols has generally eluded hospitals. For example, in a systematic review of 96 studies from around the world, Erasmus et al. reported a median hand hygiene compliance rate of 40% in hospital units of all kinds.[2]In 2005 the World Health Organization announced the launch of its first Global Patient Safety Challenge, which was focused on improving hand hygiene.[3]

In this project, we describe the most important specific causes of hand hygiene noncompliance at their respective settings and subsequent targeting of specific interventions to remedy them.

II. Material and Methods

This prospective Hand Hygiene Project was a 6 month Quality improvement project(June 2018 to November 2018) designed to improve hand hygiene compliance at Vishwaraj hospital. Data on hand hygiene compliance were collected monthly from June 2018 to November 2018. Because this project involved increasing compliance with already existing hospital policies and because all staff was expected to comply, Institutional Review Board approval was not required.

Project Planning and Implementation

The project followed the general outline of a typical, five-step Six Sigma project (Define, Measure, Analyze, Improve, Control)— Define the problem precisely, Measure its magnitude reliably, Analyze the causes of hand hygiene failure, Implement interventions targeted to specific causes, and Control (sustain) the improvements over time.[4] The teams paid close attention to change management throughout the project. Specific tools were used to engage all relevant stakeholders in the project, identify particular sources of resistance to change to facilitate overcoming them, maintain support for the interventions, and hand over oversight of the improvements to frontline staff to facilitate their sustainability

Defining and Measuring Hand Hygiene:

- Hand hygiene was defined as washing (or cleaning) hands with an alcohol- based foam or gel or soap upon entry and exit of a patient care area or environment.

Data collection—

Determining how data was collected was critical to the project was critical to the project. We realized if people know they are being monitored, they will adjust their behavior accordingly. Our team decided it was important to have secret observers collecting baseline data.

In this project, information was gathered by using:

a) Hand hygiene observers

b) Just- in- time coaches.

Rather than just collecting compliance information, the hand hygiene observers collected data on the observed factors that can lead to hand hygiene non- compliance such as health care personnel entering a room with their hands full of supplies. The just- in- time coaches began data collection two weeks after the observers have begun collecting compliance data, since this effort can be considered a form of intervention. Coaches approached the health care worker when a non- compliant event occurred to gather non- observed factors of hand hygiene failures such as the perception of the health care workers that hand hygiene was not required.

Just- in- time coaching data was kept separate from the compliance data since staff members can become familiar with people in a coaching role, which could skew the data. The observed and non- observed factors information is what provides the information needed to generate targeted solutions. Just- in- time coaches included in charge nurses, shift in charges, supervisors, Head of department etc.

Just- in- time coaching helped in capture of the root causes of failure to wash hands. If personnel failed to wash their hands, coaches asked why and help identify observed contributing factors. Just- in- time data was kept separate from baseline observational data. Staff members can become familiar with people in a coaching role, which could skew the data.

Data was collected on hand hygiene audit forms which monitors five moments of hand hygiene.[5] It was collected twice every month for the first two months (May 2018 to June 2018) and later every week. Simultaneously causes for noncompliance were noted down by the observers and just in time coaches.Hand hygiene was observed in all ICUs and General wards throughout the Hospital

Analysis of Data

An average percentage of hand hygiene compliance was calculated each month. The mean percentage of data from June 2018 and July 2018 was compared to the mean data of the next two months (Post intervention phase I /Improve phase (Aug 2018 and Sep 2018) which was post the hand hygiene improvement interventions. The mean percentage of data from June 2018 to July 2018 was also compared to the mean data of the two months (Post intervention phase II /Control phase: Oct 2018 and Nov 2018) post hand hygiene improvement interventions with no just in time coaching. Causes of non-compliances were noted down and necessary actions taken. The study hypothesis is that the major improvement would occur in the Improve phase (and that this improvement would be sustained in the Control phase. The hand compliance percentage were compared by using Chi-square and $p \leq 0.05$ was considered statistically significant

III. Result

Hand hygiene Compliance and causes of noncompliance were studied during the period of June 2018 to Nov 2018 in general wards and intensive care units of Vishwaraj Hospital; 5 moments of hand hygiene were monitored.

Identifying Causes of Hand Hygiene Noncompliance

The data collected revealed nine main causes of hand hygiene noncompliance which are enumerated in Table 1. It was realizedthat almost all the specific causes of hand hygiene failure would require separate and distinct interventions to remediate. For example, inconvenient location of hand rub dispensers required purchasing and placing dispensers in close proximity to the entrance to patient rooms. Ensuring that dispensers are always full required the development and implementation of an effective maintenance program. Specific gaps in the knowledge and training of particular disciplines of caregivers, such as housekeepers or food service workers, required the modification of the hospitals' education and training programs for them. Changing the culture of a hospital unit so that all staff at every level of seniority and job type would feel not only comfortable but obligated to stop another individual from entering a patient room without washing hands required a solution entirely different from all the others.

Table 1 .Causes of Hand Hygiene Noncompliance
Ineffective or insufficient education
Lack of uninterrupted supply of hand rub, soap and paper towels
Inappropriate placement of dispensers or sinks
Hand hygiene compliance data not collected/reported accurately or frequently
Lack of accountability & just-in-time coaching
Safety culture does not stress hand hygiene at all levels
Wearing gloves interferes with process
Perception that hand hygiene is not needed if wearing gloves
Health care workers forget

Deployment of interventions

Causes of hand hygiene noncompliance were noted down by the observers and just in time coaches, were discussed and necessary solutions / interventions planned and implemented. The specific interventions are tabulated in Table 2

Table 2. Interventions for specific causes of hand hygiene	
Ineffective or insufficient education	• Training given multiple times • Daily bed side training given by ICN • Training Include information on infection prevention, and stress the organization wide commitment to hand hygiene, highlighting strategies deployed to reinforce compliance, such as posters • Training given at Induction • Reinforced education with just-in-time coaching.
Lack of uninterrupted supply of hand rub, soap and paper towels	• Pharmacy and stores to provide uninterrupted supply of hand rub, soap and paper towels • Nursing in charges to put indent for supplies well in advance
Inappropriate placement of dispensers or sinks	• Dispensers placed at entry ,bed side, and on dressing trolley • Sinks placed at accessible and convenient locations
Hand hygiene compliance data not collected/reported accurately or frequently	• Hand hygiene data collected more frequently i.e. every week for all units • Data collection done by staff aware of appropriate collection methods
Lack of accountability & just-in-time coaching	• Leaders worked as just-in-time coaches to reinforce compliance. • Just-in-time coaches, intervene to remind health care workers to wash their hands. • Applied progressive disciplinary action against repeat offenders.
Safety culture does not stress hand hygiene at all levels	• Make hand hygiene a habit by repeat training And reminders • Hold everyone accountable and responsible—physicians, nurses, food service staff, housekeepers, technicians, therapists.
Wearing gloves interferes with process	• Made it a process to wash hands, gown and then put on gloves through repeat training
Perception that hand hygiene is not needed if wearing gloves	• Training and education highlighting importance of hand hygiene with appropriate glove usage
Health care workers forget	• Health care workers to signal to a peer that they missed an opportunity and need to wash. • Announcement made every 2 hourly to remind for hand hygiene • Appreciation of staff for good hand hygiene practices • Application of disciplinary action against repeat offenders.

Hand hygiene compliance data

Hand hygiene compliance data was collected for a period of six months from June 2018 to Nov. 2018 (Table3). Five moments of hand hygiene were monitored and marked as action taken and missed opportunities. An increase in hand hygiene compliance is seen in improve phase starting from August 2018 and sustained during control phase. (Chi square=19.4; probability=0.002) as shown in Table 3 and Fig. 1

Table 3:Percentage Compliance of hand hygiene from June 2018 to Nov 2018						
Month	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18
Actions taken*	34	53	104	70	85	88
Missed opportunities	54	68	102	45	60	59
Total opportunities	88	121	206	115	145	147
Compliance %**	38.6	43.8	50.4	60.8	58.5	59.8

*Actions taken:Hand wash / Alcohol Hand Rub, ** Compliance %: Actions / opportunities * 100

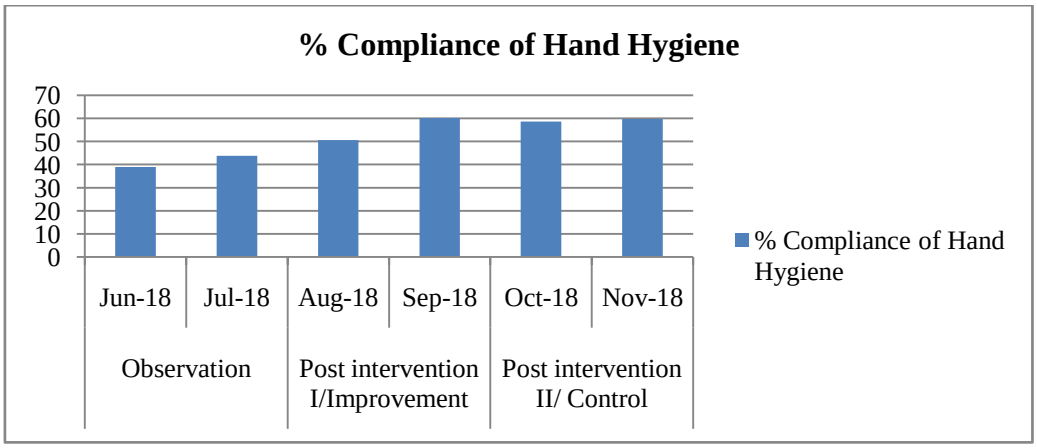

Figure 1: Percentage Compliance of hand hygiene from June 2018 to Nov 2018

We compared baseline (pre intervention) compliance data for 2 months (June 18 and July 18) to post intervention data from Aug 2018 to Sep 2018 (Improve phase) and control phase of post intervention from Oct 2018 to Nov 2018. (Table 4 and Fig. 2)

Table 4: Compliance of hand hygiene pre and post interventions

		Post intervention		
Phase	**Observation**	**Improvement**	**Control**	
Month	June 18 -July 18	Aug 18-Sep 18	Oct 18-Nov 18	Total
Actions taken*	87	174	173	434
Missed opportunities	122	147	119	388
Total opportunities	209	321	292	822
Compliance %**	41.6	54.2	59.2	

*Actions taken:Hand wash / Alcohol Hand Rub, ** Compliance %: Actions / opportunities * 100
Chi square=15.6; probability=0.000
Analysis of proportion comparison over three phases (Table 4) was statistically significant

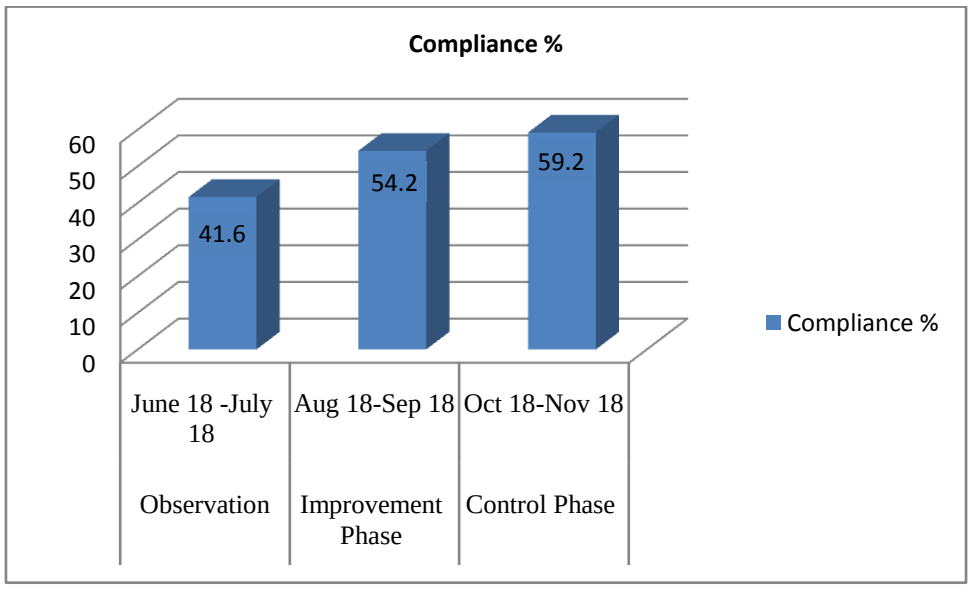

Figure 2: Percentage Compliance of hand hygiene pre and post interventions

Hand hygiene compliance during observation phase when compared with the improvement phase that is after specific interventions was found to be statistically very significant (p=0.005) as seen in Table 5. The improvement in hand hygiene compliance was sustained during the control phase (p=0.000) as seen in Table6.

Table 5: Comparison of hand hygiene compliance % during observation and improvement phase

Phase	**Observation**	**Improvement**	
Month	June 18 -July 18	Aug 18-Sep 18	Total
Actions taken*	87	174	261
Missed opportunities	122	147	269
Total opportunities	209	321	530
Compliance %**	41.6	54.2	

*Actions taken:Hand wash / Alcohol Hand Rub, ** Compliance %: Actions / opportunities * 100
Chi-square=8.01; probability=0.005

Table 6: Comparison of hand hygiene compliance % during observation and Control phase

Phase	**Observation**	**Control**	
Month	June 18 -July 18	Oct 18-Nov 18	Total
Actions taken*	87	173	260
Missed opportunities	122	119	241
Total opportunities	209	292	501
Compliance %**	41.6	59.2	

*Actions taken:Hand wash / Alcohol Hand Rub, ** Compliance %: Actions / opportunities * 100
Chi-square=15.1; probability=0.000
The percentage compliance from improvement phase when compared to control phase was not found to be statistically significant (p=0.208) which indicates that the improvement in compliance was sustained in the control phase (Table 7)

Table 7: Comparison of hand hygiene compliance % during Improvement and Control phase

Phase	**Improvement**	**Control**	
Month	Aug 18-Sep 18	Oct 18-Nov 18	Total
Actions taken*	174	173	347
Missed opportunities	147	119	266
Total opportunities	321	292	613
Compliance %**	54.2	59.2	

*Actions taken:Hand wash / Alcohol Hand Rub,** Compliance %: Actions / opportunities * 100
Chi-square=1.58; probability=0.208

IV. Discussion

Using varied methods and definitions, studies repeatedly show that improving hand hygiene compliance in hospitals reduces rates of infection. [6, 7, 8]However, efforts to improve rates of hand hygiene compliance have proved difficult to spread and sustain. [9, 10]A number of factors have been identified as barriers to such efforts. [11, 12]

We employed Lean, Six Sigma, and change management tools to systematically assess causes of hand hygiene noncompliance and to drive improvement in our hospital. The hand hygiene compliance improved and sustained the improved levels for 4 months. Our findings suggest that several factors were associated with this success and may be important considerations in the design and implementation of such programs. Each one of these different causes of hand hygiene noncompliance pointed toward very different remedial measures. Each intervention targeted to one of the most important causes of hand hygiene failure in the facility.

This study has a few limitations. Only In patients wardstaff were studied, we did not monitor outpatient staff. Also the staff was not the same every time we did the audit. Because the evaluation design compared baseline with post intervention rates of compliance, we cannot be certain that the interventions developed by project participants were solely responsible for the improvements in hand hygiene compliance. For example, we cannot exclude the possibility that influences external to this project, such as other programs or activities to reduce the frequency of health care–associated infections, played a role in influencing rates of hand hygiene compliance. Nor can we assess which of the specific interventions had the greatest effect on improving hand

hygiene because multiple interventions were deployed at the same time during the Improve phase. Finally, although we have documented substantial improvements in hand hygiene compliance, we have not evaluated that health care–associated infection outcomes concomitantly improved for these hospitals.

V. Conclusion

At baseline, hand hygiene compliance averaged 41.6% in our hospital. Data revealed nine different causes of hand hygiene noncompliance; we developed and implemented specific interventions targeted to causes of hand hygiene noncompliance. The improvements were associated with a increase in compliance from 41.6 % to 54.2 % (p<0.05), a level of performance that was sustained for 4 months through the end of the project period.

In this project we used Lean, Six Sigma, and change management tools to improve their hand hygiene compliance from 41.6 % to 54.2% and sustained that high level of improved performance for 4 months. We targeted the most important causes of hand hygiene failure. Such a targeted approach isan effectiveand efficient improvement strategy

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Fatal empyema thoracis due to *Nocardia asteroides* in a neonate

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Abstract:
A 40 day old male infant presented with fever, cough, breathlessness and refusal to feed for two weeks. X-ray chest showed right lung consolidation. Computed Tomography (CT) scan of chest revealed bilateral lung consolidation. Frank pus aspirated from pleural tapping showed Gram positive thin branching filaments. The specimen was processed on Blood agar and Lowenstein – Jensen medium. The colonies grown were identified as Nocardia asteroides by microbiological techniques. After seven days of admission patient deteriorated. CT scan showed multiple brain abscesses. In spite of extensive medication, patient did not improve and finally succumbed to infection. Nocardiosis is an uncommon infection which occurs infrequently in children. Hence presenting this case due to its rarity.

Key Word*:Nocardia, empyema ,nocardiosis*

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I. Introduction

Nocardia asteroides is an aerobic Gram positive filamentous bacterium present worldwide. Nocardiosis is an uncommon infection, which occurs infrequently in children. The disease mainly occurs as a secondary infection in patients with chronic granulomatous disease and impaired CMI such as HIV disease, recipients of organ transplants, those receiving corticosteroids and chemotherapeutic agents. [1].

There are very few cases of nocardial infections in neonates in the world literature, hence this case was reported due to its rarity.

II. Case Report

A 35 day old male infant was admitted with fever, cough, breathlessness and refusal to feed for two weeks. Fifteen days back patient had fever, cough, breathlessness, which was treated in a private clinic with no improvement. Patient was given piperacillin – tazobactum and monocef. He also had right sided umbilical discharge for the past 10 days and history of ingestion of cow's milk. The child was full term, 2.8 kg neonate, delivered by normal labour with no antenatal and perinatal complications and was immunized till date.

Physical examination revealed body temperature of 38°C, pulse rate of 100/min. On chest examination air entry was decreased on right side. Crepitations were felt on right side of chest. Umbilical discharge was present. There was no history of bleeding from any site. Few pigmented patches were noted on trunk. Rest of the physical examination was within normal limits.

Laboratory investigations disclosed a Haemoglobin of 11.8 mg/dl, Total leucocyte count of 14,600/cumm with 55% neutrophils, 40% lymphocytes, 3% eosinophils and 2% monocytes and adequate platelets. Blood culture revealed no growth. The chest X-ray showed right sided consolidation (Fig.1) USG Chest showed 3.8 x 3.5 cm lesion in right apical lobe with mobile interechoes with mild central vasculature suggestive of abscess. CT scan chest also showed right sided consolidation. Gram stain of frank pus aspirated from USG guided pleural tap showed Gram positive branching filaments (Fig. 2); which were acid fast by modified ZN staining(1% H_2SO_4) . The specimen was inoculated on blood agar (Fig 3), Lowenstein - Jensen medium. (Fig 4) Colonies grown were identified as *Nocardia spp*. by Standard microbiological tests.[2]

Speciation of *Nocardia* was done by antibiotic susceptibility testing using Gentamicin (10µg), Tobramycin (10µg), Amikacin (30µg), Erythromycin (15µg) discs after matching the suspension with 0.5 McFarland standard. But since there was poor growth on Mueller Hinton agar, blood agar was used. Plates were incubated at 35°C in air for 72 hours. Zone diameters were measured and interpreted as gentamicin ≤ 15mm, tobramycin ≤ 20mm, amikacin≤ 20mm, erythromycin ≤ 30mm. Species was identified as *N.asteroides* bythe algorithm given by Kiska et al.[3]

Inspite of isolating *Nocardia spp*. the patient was not investigated for chronic granulomatous disease due to short period of admission to hospital. The patient was given 15 mg/ kg/ day of Trimethoprim and 75

mg/kg /day of Sulfamethoxazole orally. Patient was also given Imipenem. ICD was put for drainage of pus. Patient did not improve after seven days of treatment. Hence ICD drained pus was resent for microbiological examination which again grew *N.asteroides.* CT brain showed multiple (four) brain abscesses. Largest measuring 2.1 x 1.8 x 2.1 cm in right parafalcine frontal region adjacent to frontal horn of lateral ventricle.(Fig. 5). Patient worsened and finally succumbed to infection after 11 days of admission. No obvious risk factors were detected in this case.

III. Discussion

Nocardia spp. can cause cutaneous, pulmonary and disseminated infections. Invasive and pulmonary infections mainly occur in immunocompromised host.[1]Haematogenous dissemination may occur from the lungs to the brain, kidney, liver and all organs of the body. Suppression of cellular immunity and chronic granulomatous disease(CGD) are important risk factors for Nocardia infection.[1] This patient had an uneventful antenatal, natal and postnatal history. Johnson et al had reported first case of *N.asteroides*in 1989 USA in 1 month old neonate. In this patient responded well to TMP-SMX.[4]

Drug of choice for Nocardial infections is TMP-SMX parenterally or orally. In invasive cases a combination of amikacin and imipenem with cefotaxime and TMP-SMX display synergy. Immunocompetant patients with pulmonary or systemic nocardiosis should be treated for at least six months and those with CNS involvement for 12 months.

Nocardia are extremely rare as lung pathogens in neonates, but can be easily diagnosed by modified AFB stain, culture, physiological, biochemical and susceptibility testing.

Therefore, it is important to consider nocardia infection in the differential diagnosis of children disseminated infections.

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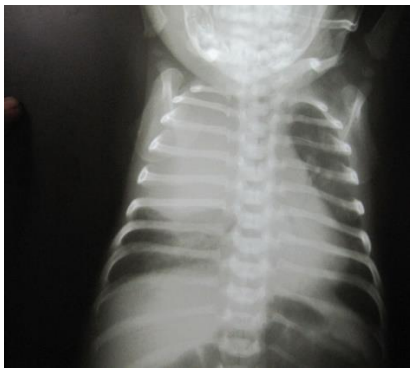

Fig.1: Xray Chest showing right sided consolidation

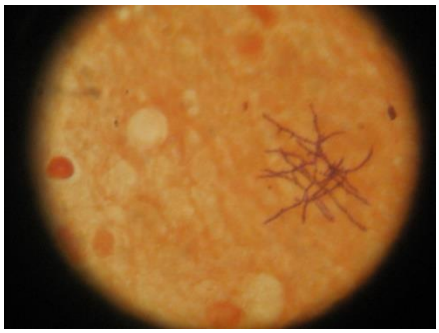

Fig.2: Gram stain showing Gram positive filaments

Fig.3: Growth on Blood agar

Fig.4: Growth on LJ medium

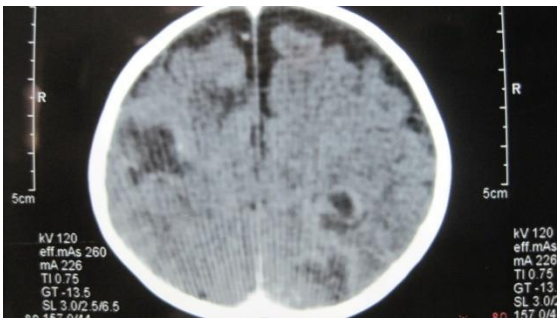

Fig.5: CT Scan showing brain abscess

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Original Research Paper Medical Science

Comparative Staining Methods for Microscopic Diagnosis of Malaria

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ABSTRACT

Objectives: The gold standard for laboratory diagnosis of malaria has remained microscopy of blood smears. Present study compared Leishman and Fields stains with Giemsa staining method for detection of malarial parasites in peripheral smear.
Methods: A prospective study was carried out at a tertiary care hospital and included 200 blood specimen from patients, clinically suspected of malaria. Blood (2ml) was collected in EDTA bulb. Peripheral smears were prepared and stained with Leishman and Field stain and compared with Giemsa stain.
Results: Malarial parasites were detected in 132 of 200 patients in peripheral smears. Leishman stain gave 100% sensitivity and specificity. Fields' stain gave 98% sensitivity and 100% specificity and was found to be the most rapid and convenient staining method.
Conclusion: Giemsa and Leishman's stains are the method of choice for staining peripheral smears. However, Fields' stain was found to be the most rapid and convenient method.

KEYWORDS Malaria, Leishman stain, Fields stain, Giemsa stain

Introduction:
Malaria has plagued mankind since ancient times and is still a significant threat to half of the world's population - 3.3 billion people living in 109 countries are at risk of contracting the disease. Estimates suggest that malaria afflicts between 350 and 500 million people every year.[1]

Malaria has been known since ancient times and has been diagnosed based on patient's signs and symptoms. The parasites in the blood were first seen in 1880 by a French army surgeon, Alphonse Laveran.[2,3,4] The discovery that the mosquito acted as a vector was due to the intuition of Patrick Manson. He was unable to undertake this investigation himself and persuaded Ronald Ross, an army surgeon, to carry out the work in India. In 1897, Ross saw what is now known to be the oocysts of *P.falciparum* in an anopheline mosquito.

In 1891, Romanowky introduced staining methods for these parasites.[2,3] Today more than a century later, microscopic detection and identification of Plasmodium species in Giemsa stained blood films remains the gold standard for laboratory diagnosis. The Romanowsky stains are best used to study the structural details of parasites. Several modifications are now available which are easier to use and give better results.

Aim: To compare Leishman and Field's stains for the detection of malaria parasites in peripheral smear with Giemsa staining method.

Materials and methods:
The study was carried out at a tertiary care hospital and included 200 blood specimen from patients clinically suspected of malaria. 3ml of blood was collected as per protocol in an EDTA bulb. Three thick and thin peripheral smears were prepared and stained with Leishman and Fields stain and compared with Giemsa stain as per standard protocol.[5,3] Readymade Giemsa, Leishman, Field's A and B stains from HiMedia Laboratories Pvt. Ltd. were used. Speciation of the plasmodium species was done from the thin peripheral smear. Data were analyzed in SPSS. Proportions were compared using the Chi – square test.

Results:
Of the 200 samples processed in the laboratory, 132 had positive blood films.

Field's stain was negative in 3 cases which were positive by Giemsa and Leishman stain (table 1). Leishman stain gave 100% sensitivity, specificity, PPV and NPV when compared with Giemsa staining. Field's stain gave a lower sensitivity but 100% specificity (table 2). Field's stain was rapid and convenient for both thick and thin smears (table 3)

Discussion:
Three stains were compared; Giemsa, Leishman, and Field's. Giemsa stained thick smear was taken as the gold standard. Thin smear was used for species identification. In present study when Leishman stain was compared with Giemsa, it gave100% sensitivity and specificity (Table 2). Leishman stained thick films are considered to be the gold standard in malaria diagnosis.[6] Giemsa staining is the most commonly used method for both thin and thick films all over the world for the quality of the stain and of greater importance, its stability in tropical climates.[7] Some laboratories prefer Leishman stain as it is alcohol based and simultaneous fixing and staining occurs.[8] The staining quality of Leishman stain is excellent.[8,7] In the tropics care needs to be taken in storing Leishman stain as evaporation of alcohol may concentrate the stain and alter the staining time. The main disadvantage in using Giemsa and Leishman stain is that the staining process is time consuming. This can be overcome by using rapid Field technique.[8] Fields staining is a good method to stain thick films and is extremely quick.[7]

In the present study Field's stain failed to pick up three cases of malaria (Table 1). It had a sensitivity of 97.72 % and a specificity of 100 %. Mendiratta et al when evaluating different methods for diagnosis for *P.falciparum* malaria compared Leishman and Field's stain. Out of the 443 subjects examined for *P.falciparum*18.28% were detected by Leishman stain and 6.32 % by Field's stain. Field's stain missed 53 (65.4%) cases. All smears positive by Field's stain were positive by Leishman stain. The sensitivity of Field's stain was found to be low (34.57%).[9] Similarly when Lema et al have compared 5 methods of malaria detection in an outpatient setting which also included staining methods, they observed 82-98 % sensitivity and 85-99% specificity, 67 – 98 % PPV and 97-99% NPV for Giemsa stain. They had 86-98% sensitivity, 94-100 % specificity, 67-98% PPV and 97-99 % NPV for Field's stain. They found that staining for Giemsa took longer. However on the basis of sensitivity, specificity, convenience and cost they thought that Field's stained thick blood film remains the most appropriate method for diagnosis of *P. falciparum* in health facilities.[10]

The present study shows that Leishman stain is comparable to Giemsa in sensitivity and specificity. Fields stain gave 97.72% sensitivity and 100% specificity. But convenience in using the Fields staining method, stability of its reagent in tropical countries, shorter duration of staining method makes it an appropriate method to be used in conditions where a large number of slides need to be stained and interpreted (Table 3). Determining a good staining method which is rapid, cost effective, gives consistent results and can be used both by experts and novices is the key to an effective diagnosis.

Conclusion:

In the present study, Leishman stain gave 100 % sensitivity and specificity when compared to Giemsa stain. Field's stain had a sensitivity of 97.72 % and specificity of 100%. Giemsa and Leishman stain gave excellent results. Although Field's stain showed a slightly decreased sensitivity compared to Leishman and Giemsa stain it was found to be the most rapid method especially when a large number of slides needed to be processed.

TABLE 1: RESULTS OF GIEMSA, LEISHMAN AND FIELD'S STAIN (N=200)

Stain	Positive for malaria	Percentage positivity
Giemsa	132	100
Leishman	132	100
Field's	129	97.72

TABLE 2: COMPARISON OF SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE (PPV), NEGATIVE PREDICTIVE VALUE (NPV) OF LEISHMAN AND FIELD'S STAIN (N=200)

Stain	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leishman	100	100	100	100
Field's	97.72	100	100	95.77

TABLE 3: COMPARISON OF GIEMSA, LEISHMAN AND FIELDS STAIN

	Giemsa	Leishman	Fields
Fixation of thin smear required	Yes	No	Yes
Dehaemoglobinisation of thick smear required	Yes	Yes	No
Time required for staining	30 minutes	15 minutes	<1minute
Percentage positivity	100	100	97.72

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Liver abscess caused by *Acinetobacter lwoffii*

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Abstract:
We report a case of pyogenic liver abscess who presented with fever generalised body weakness, and pain in the abdomen. On examination patient had tenderness in right hypochondriac region . Ultrasonography of the abdomen showed enlargement of the liver with features suggestive of abscess in the right lobe and left lobe. Ultrasound guided liver aspiration was done. Gram stain from the pus sample showed presence of Gram negative coccobacilli. Pan sensitive Acinetobacter lwoffii grew in culture. Patient recovered well after intravenous antimicrobial therapy. Acinetobacter lwoffii is emerging as a pathogen in both hospital and community settings.

Key Word*: Acinetobacter lwoffii, pyogenic liver abscess*

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I. Introduction

Acinetobacter lwoffii, a nonfermentative gram-negative aerobic bacillus, which presents in the normal flora of the oropharynx and skin, has recently been reported as a cause of human infection in patients with impaired immune system.[1] Herein, the authors present a case report of pyogenic liver abscess in an otherwise healthy adult. There are very few cases of liver abscess due to *Acinetobacter lwoffii*, hence this case is reported due to its rarity.

II. Case Report

A 66-year-old man, presented to the casualty of our hospital with the complaint of high grade fever, generalised weakness since last one week. Patient also gave history of pain in abdomen which was usually located in the right hypochondrium since last one week. There was no history of diabetes or any other co morbidity. No history of tuberculosis or similar episode in the past. No relevant family history was present.

On examination he was conscious, febrile (Temperature-100.4 °F), pulse rate 90/min and blood pressure were 90/60 mmHg. He had pallor and mild icterus. On per abdominal examination there was tenderness in right hypochondriac region. Rest of systemic examination was within normal limit. Laboratory investigation revealed hemoglobin 10.2 gm/dl, total leukocytes count 12000/cumm, (polymorphs 82 %, lymphocytes 10%, eosinophil 1%), Platelet count of 3.79 lacs/cumm, liver enzymes (SGOT/SGPT-80/101), coagulation profile (PT/INR-13.10/1.09) and Alkaline phosphatase was 187 IU. Renal function and serum electrolytes were within references range. Serology for Hepatitis B surface antigen, HIV and other hepatitis markers (HCV) were non-reactive. Ultrasonography of the abdomen showed enlargement of the liver with features suggestive of abscess measuring 9.0cm×7.0cm×6.1cm and volume 200CC in the right lobe and a similar lesion measuring 6.6.cm x 3.4 cm x 5.8 cm was seen in left lobe. The findings were confirmed on CT scan.

Ultrasound guided liver aspiration was done. About 30ml of pus was sent for microbiological investigations, for culture and sensitivity. The pus sample received was processed as per standard microbiological protocol. A wet mount of pus was negative for Trophozoites of *Entamoeba histolytica*. Ziehl–Nelsen was negative for acid fast bacilli. Direct Gram's stain showed Gram negative cocci and pus cells [Fig-1].Smooth opaque colonies of around 2–3mm diameter grew on blood agar and non-lactose fermenting colonies on Mac-Conkey agar. The isolate was identified as *A.lwoffii* on the basis of biochemical reactions with cytochrome oxidase (negative), oxidative/fermentative glucose (negative), nitrate reduction (negative).It was confirmed by automated technique with Vitek-2 system. It was found to be susceptible for Amikacin, tobramycin, gentamicin, cefotaxime, cefepime, piperacillin tazobactum, carbapenems, quinolones and colistin. The patient was given injectable ceftriaxone, Amikacin and Metronidazole. Patient recovered well and was discharged on sixth day.

III. Discussion

The most common pathogens associated with Pyogenic liver abscess are *Escherichia coli, Klebsiella pneumoniae, Bacteroides, Enterococci, Streptococci, and Staphylococci*.[2] *Acinetobacter lwoffii* was first reported to be causing pyogenic liver abscess in 2016 by Singh et al [1].The patient was a diabetic and was on irregular anti hyperglycaemic drugs. In 2018 Mohanty et al presented a case of liver abscess by *A. lwoffii* in an immunocompetentpatient patient , however the patient was an alcoholic which could have contributited in to lowering his immune status. [3] *Acinetobacter* species infections tend to occur in patients with chronic diseases as diabetes mellitus, chronic obstructive pulmonary disease, renal disease, heavy smoking, and excess alcohol consumption. [4]Here we have presented a rare case of pyogenic liver abscess caused by *A. lwoffii* in an adult with no obvious co morbidities.

Acinetobacter species were considered as low pathogenic during 1960s but with the introduction of powerful new antibiotics in clinical practice and agriculture and the use of invasive procedures in hospital intensive care units (ICUs), drug resistant-related community and hospital-acquired *Acinetobacter* infections have emerged with increasing frequency. [5] Among its species, *A. baumannii* has emerged as of a greatest clinical importance and is associated with hospital outbreaks. But infections due to other species like *A. lwoffii* have also been reported in hospitals and community settings.[6,7] Due to its ubiquitous nature, it is a potential opportunistic pathogen in individuals with impaired immune system, and it has been identified as a cause of nosocomial and community acquired infections like septicaemia, bacteraemia, bacteriuria, pneumonia and endocarditis.[1]However our patient had no history for previous hospitalisation , intake of higher antibiotics or co morbidities

Our strain was found to be pan sensitive but many studies have reported the high rates of antibiotic resistance in *Acinetobacter* species. Mittal et al., reported high resistant to imipenem (57%), cotrimoxazole (57%), gentamicin (82%), piperacillin + tazobactum (61%) in *A. lwoffii* as compared to other non-baumanii *Acinetobacter* spp in nosocomial infections.[6] *Acinetobacter* species has been known to produce variety of beta – lactamases which confer resistance to aminopenicillins, ureidopenicillins, narrow-spectrum and expanded-spectrum cephalosporin, cephamycins. Partial susceptibility is retained for some relatively new antibiotics such as broad-spectrum cephalosporin (cefotaxime, ceftazidime, and cefepime), tobramycin, imipenem, amikacin, and fluoroquinolones.[8] Since our patient did not recall any previous history of hospital stay or prolonged antibiotic intake and our strain was pan sensitive , we presumed it to be community acquired infection.

IV. Conclusion

This is the rare case report of community acquired liver abscess caused by *A. lwoffii*. Apart from *A. baumananii* other species are also emerging as pathogens in both hospital and community settings. Preventive measures need to be taken to halt the emergence of new drug resistant species.

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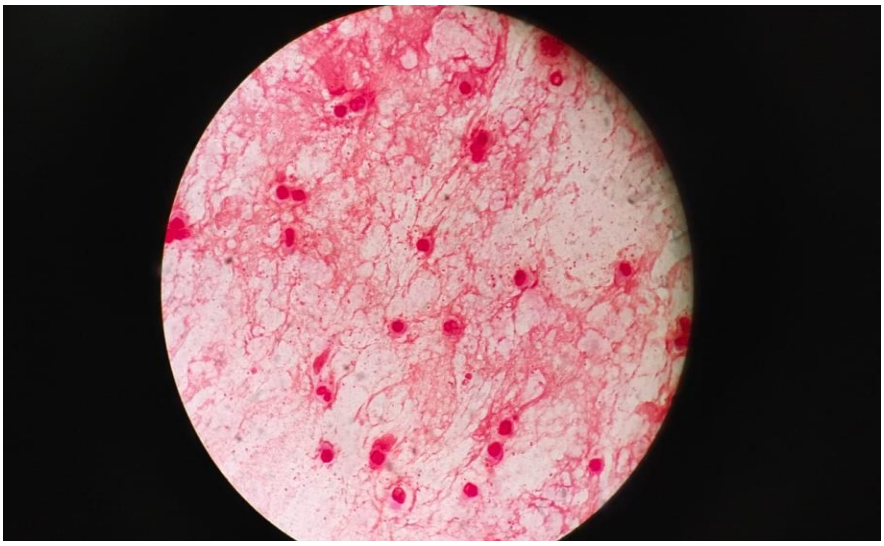

Fig.1 : Gram stain showing pus cells and Gram negative cocci.

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Simple educational intervention used to improve hand hygiene compliance

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Abstract:
Background*: Proper hand hygiene is the single most important, simplest, and least expensive means of reducing the prevalence of Health care associated infections and the spread of antimicrobial resistance. The purpose of this project was to observe compliance of hand hygiene, study improvement in hang hygiene compliance and infection control rate improvement following educational intervention*
Materials and Methods*: This was a hand hygiene program implemented with the hopes of improving hand hygiene and decreasing hospital-associated infection rates. A multidisciplinary group developed a hospital supported campaign. Opportunities for hand hygiene were observed during 2 periods, pre campaign and post campaign. The frequency of hospital-associated infection in the burns ward was tracked over time by review of records pre and post campaign*
Results*: Pre campaign the compliance for hand hygiene was 27.09%. The staff was educated regarding importance of hand hygiene and effective hand hygiene techniques. This improved the compliance and post campaign it was found to be 42.06%.The difference between compliance ;pre and post educational campaign was found to be statistically significant.*
Conclusion: *The study showed that Hand hygiene can be improved by simple educational intervention and it is an important preventive measure to reduce hospital acquired infections*
Key Word: *Hand hygiene,compliance, educational intervention*

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I. Introduction

Nosocomial infections constitute a major challenge of modern medicine. On average, infections complicate 7% to 10% of hospital admissions.[1] Over 1.4 million people worldwide are suffering from Hospital Acquired Infections or nosocomial infections as they are called. In India, nosocomial infection rate is at over 25 per cent and it is responsible for more mortality than any other form of accidental death. The irony is that one-third of all such episodes are preventable.[2] Transmission of microorganisms from the hands of health care workers is the main cause of nosocomial infections.[1]

Hand hygiene is the simplest, most effective measure for preventing nosocomial infections.[1,3,4.5] Despite advances in infection control and hospital epidemiology, Semmelweis' message is not consistently translated into clinical practice , and health-care workers' adherence to recommended hand hygiene practices is unacceptably low. Average compliance with hand hygiene recommendations varies between hospital wards, among professional categories of health-care workers, and according to working conditions, as well as according to the definitions used in different studies. Compliance is usually estimated as <50%.[2,5]

Hand hygiene effectiveness and compliance can be improved with simple educational intervention.[6,7]It is an effective means of preventing hospital-associated infection. Few studies aimed at increasing hand hygiene in the hospital setting have shown sustained improvement and concurrent decreases in hospital-associated infections.[8]

II. Material and Methods

We implemented a hand hygiene program with the hopes of improving hand hygiene and decreasing hospital-associated infection rates. A multidisciplinary group developed a hospital supported campaign. Opportunities for hand hygiene were observed during 2 periods, pre campaign and post campaign. The frequency of hospital-associated infection in the burns ward was tracked over time by review of records pre and post campaign. [4]

Handwashing facilities are conveniently located throughout the institution. Sinks are located inside every patient room, along with towels and unmedicated soap. Dispensers of hand antiseptic solutions are

available in high-risk areas. Individual bottles containing an alcohol-based preparation of 0.5% chlorhexidinegluconate are available in every ward.

The study took place in the burns ward in January –February 2011. We informed department chairs about the upcoming study in advance. Personnel were aware that they were being observed. In accordance with the requirements of the institutional review board, we did not identify staff members by unique identifier.

Potential opportunities for and actual performance of handwashing were observed randomly during day and night over 7 days. Observations could be prolonged until completion of a patient care episode. Data were recorded on a report form. Compliance with handwashing was defined as either washing the hands with water or plain soap or rubbing the hands with an antiseptic solution. Departure from the room after patient care without handwashing was regarded as noncompliance. Handwashing was required regardless of whether gloves were used or changed. Failure to remove gloves after patient contact or contact between a dirty and a clean body site on the same patient was considered noncompliance.[1]

The staff was educated through a hand a hygiene campaign which included lecture explaining importance of hand hygiene in control of hospital acquired infections, presentation, poster and slogan competition, and a demonstration explaining effective hand hygiene technique.

A total of 61 personnel working in the burns department were asked to clean their hands using an alcohol gel containing a clear fluorescent substance. They were unaware of the assessment method. Performance was assessed by examining their hands under UV light to identify areas that had been neglected. Subjects could visualize which areas they had missed and were then educated regarding hand-washing technique which clearly described an effective method of hand-washing with the gel.

Post campaign the opportunities for hand hygiene were recorded in the same ward on the similar record forms. The hospital acquired infection were calculated over a period of one month post hand hygiene campaign.[4]

Statistical analysis: Statistical analysis was done with the help of a professional medical statistician. McNemar test was applied to the data and Chi square and p value calculated.

III. Result

The present study was carried out in the burns unit of a tertiary care hospital.A retrospective analysis of nosocomial infection over a period of one month was done which found to be 9.13%.An observational study was done to find the compliance of hand hygiene in the ward. A total of 860(Pre campaign) and 718 (Post campaign) opportunities were reported as shown in Table 1 and Fig.1. Hand hygiene compliance was found to be 27.09%. In order to improve the hand hygiene an educational campaign was organized. The campaign included a lecture explaining importance of hand hygiene and its role in control of hospital acquired infections, presentation, poster and slogan competition, and a demonstration explaining effective hand hygiene technique. Post campaign the compliance of hand hygiene improved and was found to be 42.06% as shown in Table 1and Fig 2. The hospital acquired infections also reduced to 7.04% as shown in Table 2 and Fig. 3

Table I: Compliance of hand hygiene pre and post campaign

	Opportunities for hand hygiene			
	Actions taken*	**Missed opportunities**	**Total opportunities**	**Compliance** (%)**
Pre campaign	233	627	860	27.09
Post campaign	302	416	718	42.06

*Actions taken:Hand wash / Alcohol Hand Rub,** Compliance %: Actions / opportunities * 100
McNemar test was applied for the statistical analysis; Chi square =113.347 $p < 0.01$
For such a high Chi square value p value is very very low.

Pre campaign the compliance for hand hygiene was 27.09%. The staff was educated regarding importance of hand hygiene and effective hand hygiene techniques. This improved the compliance and post campaign it was found to be 42.06%.The difference between compliance; pre and post educational campaign was found to be statistically significant.There was significant improvement in compliance of hand hygiene pre and post educational campaign

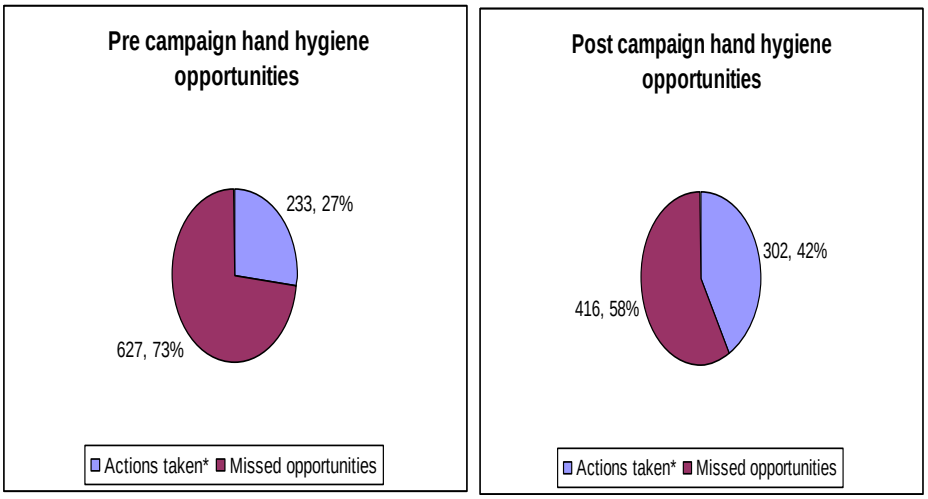

Fig 1. Hand hygiene opportunities pre and post campaign

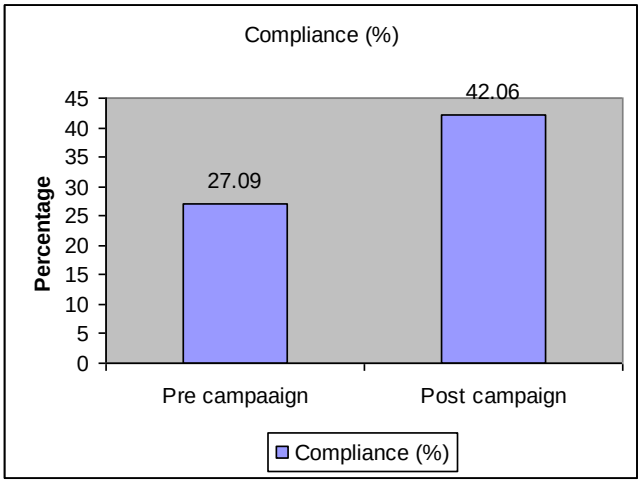

Fig 2 Compliance of hand hygiene

Table II: Hospital acquired infections pre and post campaign

	Hospital acquired infections			
	Yes	**No**	**Total**	**Percentage (%)**
Pre campaign	19	189	208	9.13
Post campaign	16	211	211	7.04

McNemar test was applied for the statistical analysis; Chi square =145.152 $p< 0.01$

The rate of Hospital acquired infections pre and post educational campaign was reduced from 9.13% to 7.04% and the difference was found to be statistically significant.

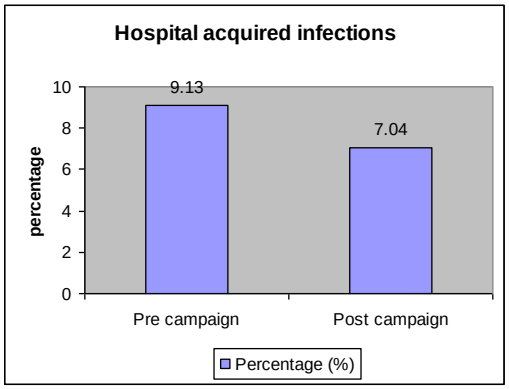

Fig 3. Hospital acquired infection pre and post campaign

IV. Discussion

It was found that the rate of nosocomial infections was high and increasing in the burns unit of our institution. One of the important strategies in prevention of nosocomial infections is limiting transmission of organisms between patients in direct patient care through adequate hand washing and glove use.[4]

Thus this study aimed at calculating the compliance and hospital acquired infection rate. We tried to improve the hand hygiene compliance through education and training. We found that with improvement in hand hygiene compliance there was significant reduction of hospital acquired infections from 9.13% to 7.04%. This reduction was statistically significant. This re-emphasizes the role of hand hygiene in control of nosocomial infections.There are innumerable studies which have tried to find the impact of hand hygiene on hospital acquired infections.[9, 10, 11]

Our study shows that the primary problem with hand washing is laxity of practice. During routine patient care, health care workers disinfected or washed their hands in approximately half of the indicated instances. Noncompliance with hand washing is a substantial problem in our hospital and is associated with identifiable factors. This suggests that interventions aimed at improving hand washing practices may be more effective if they focus on selected wards, groups of health care workers, or patient care situations. Health care workers need about 1 minute to walk to the sink, wash their hands, and return to the patient[1], the total amount of time spent washing hands becomes prohibitive. One possible solution is to replace time-consuming hand washing with bedside hand antisepsis. Whether switching from traditional hand washing to hand antisepsis improves compliance deserves testing in clinical trials.[1]

Our study has several limitations. First, although our observations were as unobtrusive as possible health care workers may have changed their behavior because they were being observed. Such a bias would probably inflate compliance estimates, a the real situation may be even worse than reported. Second, whether our results can be generalized to other health care institutions is uncertain because both the infrastructure and the organization of work influence behavior. Third the study was carried out for a short duration of time. An extensive study would be required to reach to a conclusion.

V. Conclusion

Healthcare worker's hands are the most common vehicle for the transmission of healthcare-associated pathogens from patient to patient and within the healthcare environment. Hand hygiene is the leading measure for preventing the spread of antimicrobial resistance and reducing healthcare-associated infections, but healthcare worker compliance with optimal practices remains low in most settings.We observed a improved compliance following education and training of staff. The rate of hospital acquired infections also showed a decline.

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