TAG 3`. Primers, dntps, Taq DNA polymerase, Taq buffer, $MgCl_2$ were obtained from Promega (USA). The thermocycling mix contained 0.5 μg of the extracted DNA 0.5 μM of each primer, 100 μM of each dNTP and one unit of Taq DNA polymerase, 2.5μM $MgCI_2$. The thermocycling conditions as follows: 95°C for 5 minutes for one cycle then 95°C for one minute, 57°C for one minute and 70°C for 2 minutes for 35 cycles on thermalcycler (Biorad, USA). The reaction then incubated at 72°C for 10 minutes. Ten microliters were removed from the amplified mixture and subjected to ethidium bromide agarose gel electrophoresis (1.5% agarose) at 70 Volts for one hour along with 100 bp stepladder (Poromega). Agrose gel then stained, visualized under ultra-violet light and photographed by photo-documentation system (Vilber Lourmat).

The presence of meca gene was tested for 102 *Staphylococcus aureus* and 96 coagulase negative Staphylococcus disc diffusion fails to detect 6/26 MRSA and 3/24 MRCON. This test also identifies 4/76 MSSA as MRSA and 1/72 MSCON as MRCON. The sensitivity, specificity and accuracy of disc diffusion was 76.9%, 94.7% and 85.8% for *Staphylococcus aureus* and 87.5%, 98.6% and 93.1% for coagulase negative Staphylococcus **(Table 1)**. The discrepant MSSA (6/26) and MSCON (3/24) were retested by broth microdilution assay. The results showed that 4/6 MSSA and 2/3 MSCON had minimum inhibitory concentrations (MIC) of more than 8μg/ml for methicillin and therefore had posses the methicillin resistance gene. The other 3 were meca negative and had their MIC 0.5μg/ml indicating sensitivity to methicillin. The other discrepant MRSA 4/76 and MRCON 1/72 strains were also retested by microdilution assay and their MIC to methicillin were 4 and 8 μg/ml (borderline resistant). They were also retested for amplified meca gene and showed PCR product (997bp) upon retesting.

Methicillin resistant Staphylococci are one of the most common causes of nosocomial infections. Standard bacterial identifications and susceptibility testing frequently require as long as 72 hours and there may be a difficulty in identifying methicillin resistance due to the heterogeneous nature of resistance to methicillin. Disc diffusion fails to detect 6 MRSA and 3 MRCON while their resistance was detected by PCR assay through meca gene amplification. Microdilution in Muller-Hinton broth for these 9 discordant strains showed that 4/6 and 2/3 were methicillin resistant and three were methicillin sensitive. The last 3 were mecA positive, these may have a nonfunctional meca gene or a nonactive PBP2a protein. This also indicate that disc diffusion assay has the lowest sensitivity compared to microdilution and PCR assay in detecting methicillin resistant. Five mecA negative (4 MRSA and 1 MRCON) were resistant to methicillin by disc diffusion assay. Minimum inhibitory concentrations of microdilution showed that all isolates have a borderline resistance (MIC 4-8 μg/ml). These resistant strains should be retested for possibility of β-lactamase over production 2 or for modification of normal PBP2agene.[4]

This study shows that the molecular assay for mec A by PCR should be considered as a gold standard for detection Staphylococcal methicillin resistance as reported previously.[1,2,4,5] Negative mecA may show methicillin resistance and positive mecA may be sensitive[2,4] so a combination of both molecular and microbiological may be the best for detecting methicillin resistant *Staphylococcus species*.

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Osmotic fragility and Na^+-K^+ ATPase activity of erythrocytes of HIV/AIDS patients

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Symptomatology of human immuno-deficiency virus/acquired immuno-deficiency syndrome (HIV/AIDS) is very diverse. However, anemia is one of the most universal clinical symptoms of the disease. The etiology of anemia in HIV disease has been extensively researched primarily from the

physiological and pharmacological angles. Malnutrition in HIV/AIDS has also been widely reported.[1,2] Nutrient deficiencies can result in disruption of supramolecular structures like biomembranes. We therefore decided to investigate the osmotic fragility of erythrocytes of HIV/AIDS patients. Since the sodium$^+$-potassium$^+$ adenosine triphosphatase (Na^+ -K^+ ATPase) is the major transmembrane pump involved in regulating osmosis in the cell; we considered it pertinent to determine its activity in the erythrocytes of HIV/AIDS patients. This was a cross-sectional study involving 56 HIV seropositive subjects recruited and confirmed positive at the Nigerian Institute of Medical Research (NIMR) Human Virology Laboratory in Lagos, Nigeria. Confirmations were carried out with the Genie II HIV Confirmation kit. Participants who were on multivitamins, mineral supplements, or cardiac glycosides, or both, or prolonged non-AIDS related treatment were excluded from the study. Some minerals have been reported to reverse osmotic fragility in erythrocytes and cardiac glycosides are specific inhibitors of Na^+-K^+ ATPase activity. The HIV positive group was subdivided into 2 groups: those who were on antiretroviral therapy (ARV) at the Institute and those who had not commenced any form of antiretroviral therapy (non-ARV). The HIV positive subjects were grouped either as ARV (35 persons) or non-ARV (21 persons) subjects. Ten HIV seronegative persons served as the control group. The age range was between 20 and 60 years. Blood samples were collected by venous puncture into potassium EDTA bottles. Four ml were aliquoted for $CD4^+$ T-lymphocyte count within 6 hours of collection using the Dynabeads method; one ml was aliquoted for erythrocyte ghost membrane (EGM) preparation while the remaining one ml was used for osmotic fragility assay. Two ml of blood were taken from the control subjects since $CD4^+$ counts were not conducted on them. All tests were conducted on the day of collection. The HIV disease-stage classification was according to the Centers for Disease Control revised 1993 classification for HIV infection among adolescents and adults. Osmotic fragility of the erythrocytes was determined by using saline solutions of 0.85%, 0.65%, 0.35%, and 0.1% Nacl to which, 20µl of freshly collected blood was added. The absorbance of the supernatant was read at 583nm. One ml of freshly collected whole blood was used for the erythrocyte ghost membrane preparation. Sodium$^+$-potassium$^+$ adenosine triphosphatase activity was calculated as the difference between total ATPase activity and ouabain-inhibited activity. The total ATPase activity was assayed in an incubation medium consisting of 50mM Tris-HCl (pH 7.4), 120mM NaCl, 20mM KCl, 4mM MgCl2, 240mM sucrose, 1mM EDTA and 3mM disodium ATP. Fifty µl of EGM suspension were aliquoted into 2 tubes labeled one and 2. One hundred µl of incubation medium were also added to each tube but 100µl of one mM ouabain solution was added to tube 2 only. The reaction mixtures were incubated at 37^{o}C for 20 minutes. They were stopped by adding 100µl of one percent SDS. Inorganic phosphate produced from the hydrolysis of ATP by ATPase and protein concentration of the EGM was determined.

Table 1 - Degree of hemolysis of red blood cells and Na+ -K+ ATPase activities of HIV negative (control), non-ARV and ARV HIV/AIDS in 0.65% saline solution.*

Subject	Percent Hemolysis	Na+ -K+ ATPase activity (nmol Pi/h/mg protein)
Control	0.83 ± 0.36	2.22 ± 0.81
Non-ARV	3.19 ± 1.11	3.01 ± 0.52
ARV	2.56 ± 0.81	3.69 ± 0.58

* values represent mean ± standard error of mean
Non-ARV - non antiretoviral
ARV - antiretroviral

At 0.6% hypotonic saline concentration the red blood cells of the non-ARV and ARV groups were found to have higher percentages of hemolysis than the control group. **(Table 1)** The non-ARV group had a greater degree of red blood cell fragility than the ARV group. Both groups showed statistically significant results. ($p<0.05$) The Na^+-K^+ ATPase activities for the non-ARV and ARV groups were higher than the activity of the control group. **(Table 1)** The differences were however, not statistically significant. ($p<0.05$) On detailed investigation the Na^+-K^+ ATPase of non-ARV and ARV subjects with $CD4^+$ T-lymphocyte count of less than 200 cells per microliter of blood showed higher activity than subjects with $CD4^+$ counts of between 200-499 cells per microliter.

Osmotic fragility has been associated with lower concentration of protein sulfhydryls in erythrocyte ghost membranes.[3] Xia et al[3] suggested in their report that an important function of zinc is to protect cysteine residues in critical plasma membrane proteins from auto-oxidation. Auto-oxidation of the cysteine residues will ultimately lead to a significant conformational change in these proteins, which may in turn cause structural fragility of the plasma membrane. Micronutrient deficiency has been reported in AIDS patients.[1] The deficient micronutrients include zinc, vitamin A, iron, iodine, and trace elements.[2] These nutrients are essential as cofactors for the proper functioning and structural integrity of various biomolecules – especially proteins. Some act as antioxidants. A deficiency in a critical micronutrient can completely upset the homeostatic functioning of a cell or the entire organism. It could be attributable to reduced intake, increased utilization[4] or urinary excretion.[5,6]

The report that reduced micronutrient intake may lead to nutritional deficiency lends credence to osmotic fragility of red blood cells observed in the HIV positive subjects. Anorexia, nausea, vomiting, and diarrhea are conditions that can result in reduced nutrient intake, which leads to malnutrition. These secondary symptoms have been observed and reported in acute and late stage HIV disease.

It may seem surprising that patients who are undergoing antiretroviral therapy, and are showing significant improvement in health, should also be significantly susceptible to osmotic stress. These patients may have osmotically fragile cells also as a result of micronutrient deficiency. Stephensen et al[5] and Jordao et al[6] reported that deficiency might occur in HIV/AIDS as a result of increased urinary excretion. Antiretroviral therapy involves a cocktail of drugs taken under a strict regimen. In an attempt to detoxify or metabolize, or both, these drugs the liver increases their water solubility. Ultimately, there is an increase in urine production and a depletory loss of vital water-soluble nutrients like the metallic ions may occur. The patients may therefore suffer from conditions like anemia. Zidovudine (AZT) therapy has been reported to be the most frequent cause of anemia in HIV-infected persons. This supports our findings of a possibility of anemia in patients on antiretroviral therapy. Whereas previous works have attributed this condition to marrow erythroid hypoplasia, aplasia, and megaloblastic maturation, we believe that from the present data and cited literature, osmotic fragility resulting from a micronutrient deficiency is critical to the development of anemia in antiretroviral therapy patients. Sodium$^+$-potassium$^+$ adenosine triphosphatase activities of the erythrocytes were found to be increased in non-ARV and ARV HIV positive patients compared to HIV negative patients, though they were not statistically significant in the present study. The increased activities may be a consequence of the osmotic fragility of the plasma membrane of the erythrocytes discussed above. The Na^+-K^+ ATPase pump is the primary mechanism by which the cell prevents lysis from osmotic stress. The activity of the pump increases when the cell is threatened with plasmolysis. The pump performs a continual surveillance role in maintaining normal cell volume. The Na^+-K^+ ATPase activities increased with the degree of severity of the disease as measured by the $CD4^+$ counts. For both the ARV and non-ARV groups the average Na^+-K^+ ATPase activity of those with $CD4^+$ counts of less than 200 cells/μl of blood was higher than those with $CD4^+$ counts of between 200-499 cells. Further, the activities of the ATPase in the 2 $CD4^+$ count classifications (200-499 and less than 200 cells/μl) were higher for the untreated HIV/AIDS subjects than the treated subjects (the non-ARV and ARV subjects).

Data shows that the Na^+-K^+ ATPase activity of HIV/AIDS persons was slightly elevated with increased severity of the disease. This corroborates the preceding finding that the erythrocytes of HIV/AIDS persons are highly susceptible to osmotic stress and greater so when the disease is left untreated. The plasma membrane becomes highly porous to trans-membrane cationic movement. Cations like Na^+ and K^+ move down their concentration gradients. In an attempt to reverse the resultant hypernatria of the intracellular fluid the Na^+-K^+ ATPase activity is increased.

In conclusion, data obtained from the present study indicate that osmotic fragility of erythrocytes is significantly increased in HIV disease. The Na^+-K^+ ATPase activity of the erythrocytes is only marginally increased in an attempt by the cells to reverse the deleterious effects of osmotic fragility in HIV/AIDS disease.

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Tonsillectomy blood splash

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Hepatitis B virus and human immunodeficiency virus (HIV) can be transmitted via certain routes including conjunctiva.[1] Tracheostomy, air drilling, local anesthetic infiltration and beside other numerous surgical procedures, tonsillectomy puts otolaryngologist