



# Evaluation of Some Clinical Chemistry Parameters of Wistar Rats Induced with *Staphylococcus aureus* from Clinically Diagnosed Pneumonia Patients

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## Research Article

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## Abstract

The effect and extent of changes in some clinical chemistry parameters associated with pneumonia caused by *Staphylococcus aureus* were investigated in serum samples of Wistar rats. A total of 1500 samples were collected randomly. The serum samples were tested for sodium, potassium, urea, creatinine and chloride by colorimetric methods. The concentrations of sodium ranged from 133.6±4.615 to 143.8±6.906, potassium values ranged from 5.78±1.26 to 8.02±0.779, urea gave a range of 10.143±0.69 to 14.444±2.404, chloride values ranged from 94.8±2.683 to 104.142±8.49, while creatinine values ranged from 0.489±0.033 to 1.7±2.404. The results indicated that *staphylococcus aureus* has a significant effect on the electrolyte balance causing hyperkalemia and decreased levels of sodium and chloride when compared with the normal range. It had a remarkable effect on creatinine level with only slight effect on urea. There is need to monitor the electrolyte levels in cases of *staphylococcus aureus* infection since alteration in the electrolyte levels could be harmful to health.

**Keywords:** Clinical Chemistry Parameters; *Staphylococcus Aureus*; Wistar Rats; Pneumonia Patients

## Introduction

*Staphylococcus aureus* is the most pathogenic specie of the genus *Staphylococcus* with a diameter of 0.5-1.5µm [1]. This gram positive coccil bacterium belongs to the family staphylococaceae which is a member of the fermicutes. They are frequently found as a commensal residing on the skin, skin glands, respiratory tract and on the nose of healthy individuals [2]. It is a non-moving small round shaped coccus found in grape like (staphylo-) clusters, hence it is called *Staphylococcus*. *Staphylococcus aureus* owes its ability to cause disease, in part, to the production of a repertoire of virulence factors that modulate its ability to colonize host/

inert surfaces, thwart host defenses, and disseminate to satellite sites [3].

*S. aureus* is a versatile human pathogen causing infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumonia, osteomyelitis, endocarditis as well as toxin mediated syndromes such as toxic shock syndrome and food poisoning [4]. *Staphylococcus aureus* is able to cause a large diversity of both benign and lethal infections in humans and animals because of a wide range of virulence factors that include various toxins and enzymes [5]. It has emerged as one of the most popular human pathogens and has become a leading

cause of hospital (nosocomial) and community acquired infections [6].

*Staphylococcus aureus* is usually a harmless colonizer most likely to be found in the nares of about one third of healthy persons. Colonization increases the risk of subsequent infection since those *S. aureus* infections are usually infected with their colonizing strain [7]. A breach of the skin or mucosal barrier allows the organism to gain access into adjoining tissues or the blood stream causing infection [8].

Antibiotics could be used for the treatment of most Staphylococcal infections; but in recent years *Staphylococcus aureus* have been found to develop resistance against some of these used effective antibiotics found. Lincosamides, macrolides, streptogramin, tetracycline, gentamycin and beta-lactams, particularly methicillin are some examples of the antibiotics used [9].

Pneumonia infection is usually caused by Streptococcus pneumonia. But *Staphylococcus aureus* is now a common cause of pneumonia, the most frequent pathogen identified with health care-associated pneumonia as well as a major cause of superinfection and mortality after influenza [10]. *Staphylococcus aureus* pneumonia is a potentially life-threatening infection caused by this human pathogen. The only therapies available to treat *S. aureus* pneumonia are antibiotics, a modality that is jeopardized by the organism's remarkable ability to acquire antimicrobial resistance. The cytotoxin, alpha-hemolysin produced by *S. aureus* is essential for the pathogenesis of pneumonia.

The primary form occurs without an extrapulmonary focus, presumably through direct inoculation to the lungs, and the secondary form results from hematogenous seeding of the lungs during endocarditis or bacteremia.

Pneumonia is a serious and rapidly progressive infection which is associated with high incidence of complications and mortality unless recognized early and treated appropriately [11]. Pneumonia is an inflammatory condition of the lung affecting primarily the microscopic air sacs known as the alveoli. Viruses or bacteria and less commonly other microorganisms, certain drugs and other conditions such as autoimmune diseases are common causes of pneumonia.

Pneumonia is sometimes referred to as the forgotten killer. The World Health Organization estimates that lower respiratory tract infection is the most common infectious cause of death in the World with almost 3.5 million deaths yearly (Geneva: World Health Organization, 2013). Together, pneumonia and influenza constitute the ninth leading cause of death in the United States, resulting in 50,000 estimated

deaths in 2010 [12].

Pneumonia is a common illness affecting approximately 450 million people a year and occurring in all parts of the world [13]. It is a major cause of death among all age groups resulting in 4 million deaths (7% of the world's total deaths) yearly [13,14]. Rates are greatest in children less than five, and adults older than 75 years. There is high risk of pneumonia in the developing world compared to developed world. Viral pneumonia accounts for about 200 million cases. In the United States, as of 2009, pneumonia is the 8th leading cause of death [15]. In the terminally ill and elderly especially those with other conditions, pneumonia is often the immediate cause of death. In such cases, particularly when it cuts short the suffering associated with lingering illness, pneumonia.

*S. aureus* may occur commonly in the environment and is transmitted through air droplets or aerosol. When an infected person sneezes or coughs, he or she releases numerous small droplets of saliva that remain suspended in air. These contain the bacteria and can infect others. Following exposure, the organism may establish itself in the nasopharynx of its host usually resulting in asymptomatic colonization. The organism can be carried for a period of weeks to months. However sometimes, the newly acquired pneumococcus invades host defensive mechanisms and causes illness [16].

Clinical chemistry, a branch of Clinical medicine that deals with body fluids, is based on the basic principle that a disease causes changes in the biochemistry of the body. It may cause either increase in concentration, or decrease in concentration of certain biochemical parameters or even may cause a different substance to appear. Hence, clinical biochemistry deals with changes in the composition of blood and other body fluids which are associated with the diagnosis of disease or monitoring the therapy [17]. The Hematological and blood clinical chemistry could aid in the determination of the health status, diagnosis and prognosis of disease [18].

The objective of the present study was to determine the ability and prevalence of *staphylococcus aureus* to induce pneumonia with a view to providing information on the chemistry parameters of clinically diagnosed pneumonia patients.

## Materials and Methods

The materials used for this experiment include autoclave, universal bottles, incubator (model: GP/50/CLAD/250/HYD), spirit lamp, measuring cylinder, Beam balance (Harvard trip 1400/1500 series), wire loop, hot air oven (model: Gp/50/CLAD/250/HYD), forceps, Pasteur pipettes, bijou bottles, latex gloves, Petri dishes, weighing balance, Wistar rats, test

tubes, beakers, McCartney bottles, spatula, 2ml syringes and needles, dissecting kits, coloured markers, mannitol salt agar, non-heparinized micro- hematocrit capillary tubes, Spectrophotometer, chloride calibrator, Timer, Centrifuge, cotton wool. The reagents used includes; nutrient broth, sterile distilled water, disinfectant, chloride reagent.

### Ethical Approval

Ethical approval with ReF No. REC/FOS/DEL/20/05 was obtained from the Delta state University Research and Ethics Committee.

### Collection of Samples

A total of 1500 sputa were obtained from clinically diagnosed pneumonia patients from the following centers; Central Hospital Benin, Stella Obasanjo Women and Children Hospital Benin, Central Hospital Sapele, Central Hospital Agbor, Central Hospital Warri, Delta State Teaching Hospital Oghara, Irrhua Specialist Hospital, University of Benin Teaching Hospital, Central Hospital Asaba, Central Hospital Yenagwa, Central Hospital Ughelli. These samples were collected into sterile universal bottles under the supervision of the Medical Laboratory Scientist.

### Preparation of Overnight Culture Broth

1.3g of nutrient broth was accurately weighed using a double beam balance which was then transferred into a beaker and dissolved with 100ml of distilled water. This was then sterilized using the autoclave at 121 for 15 minutes.

Universal bottles were washed with detergents, rinsed with sterile distilled water and autoclaved for 15 minutes at 121°C. Nutrient broth (4ml) was measured and poured into different universal bottle and allowed to cool. The universal bottles containing the broth were inoculated with *Staphylococcus aureus* isolated from different locations and labelled accordingly and allowed to grow overnight. One sterile bottle containing nutrient broth was not inoculated with *Staphylococcus aureus* to serve as control.

### Induction of Wistar Rats with Staphylococcal

Each animal was first and foremost weighed and then marked according to the label on the samples for ease of identification. With the aid of a syringe (2ml), the samples were drawn and injected into the animals intraperitoneally. Thereafter, the animals were kept in a cage for 14 days, properly fed and monitored for any observable changes.

### Blood Collection

The animals were re-weighed at the end of the 14th day after been infected with *Staphylococcus aureus*. Lithium heparin sample bottles were prepared and labelled according to the samples. Blood was then collected with the aid of a capillary tube. The tip of the capillary tube was inserted into the medial canthus which on reaching the orbital sinus of the eye, was quickly rotated.

Blood flow was by capillary action through the capillary tubes which was collected into the already labelled Lithium heparin bottles. Also, blood was collected with the aid of syringes and needles into the Lithium heparin sample bottles which was immediately mixed with the anticoagulant to prevent clotting which may affect results.

### Evaluation of Chemistry Parameters

#### Potassium (Colorimetric Method)

##### Principle

The amount of potassium was determined by using sodium tetrathynylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7mEq/L. Each test tube was labelled for both the standard, and control. A blank was taken. 1.0mL of potassium reagent was pipetted to all tubes. 0.01mL (10µl) of samples was added to the respective tubes, mixed and allowed to sit at room temperature for 3 minutes. After 3 minutes, the wavelength of spectrophotometer was set with reagent blank. The values were read and the absorbance of each tube was recorded.

#### Chloride

Chloride was determined by titration of the chloride with standard mercuric nitrate solution using diphenylcarbazone as the indicator. The chloride procedure used was a direct method based on a modification of the colorimetric method of Skeggs and Hochestrasser.

The test tubes were labelled. 1.5ml chloride reagent was pipetted to each tube. 0.01ml (10µl) sample was added to the respective tubes and mixed. The tubes were incubated at room temperature for five (5) minutes. Spectrophotometer was set to 480nm and zero with reagent blank. Wavelengths of 480-520nm may be used. Absorbance readings of all tubes were read and recorded. NOTE; Final colour was stable for thirty (30) minutes at room temperature.

#### Sodium (Colorimetric Method)

The present method was based on modifications of those first described with Mauna and Trinder in which sodium was precipitated as the triple salt. Odium magnesium uranyl acetate, with the excess uranium then been reacted with ferrocyanide, producing a chromophore whose absorbance

varies inversely as the concentration of sodium in the test specimen.

The Test tubes were labeled. Thereafter, 1.0ml of Filtrate reagent was pipetted to all tubes. 50 $\mu$ l of sample was added to all tubes and distilled water to the blank. The tubes were vigorously shaken and mixed continuously for 3 minutes. The tubes were centrifuged at high speed (1.500G) for 10 minutes and the supernatant fluids were tested as described below:

**Color Development:** The Test tubes were labelled corresponding to above Filtrate tubes. 1.0 $\mu$ l acid reagent was pipetted to the tubes. Then, 50 $\mu$ l of supernatant were added to the respective tubes and mixed. 50 $\mu$ l of color reagent were added to all tubes and mixed. Absorbance of all tubes were read and recorded.

### **Creatinine (Colorimetric Method)**

#### **Principle**

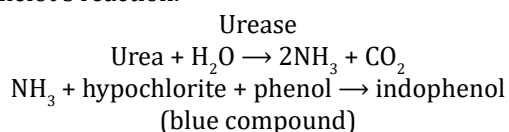
Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Fresh ddH<sub>2</sub>O was used to perform a new Gain calibration in cuvette mode. Select CREA in the Run Test screen and carry out a water blank as instructed. Mix and after 30 seconds read the absorbance A1 of the standard and sample. Exactly 2 minutes later, read absorbance A2 of standard and sample.

### **Urea (colorimetric Method)**

#### **Principle**

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.



URMD in the run screen was selected and carried out a water blank as instructed and Pipetted into test tubes. The standard and Reagent 1 were mixed and incubated at 37°C for 10 minutes.

Also, the Reagent 2 and Reagent 3 were mixed and incubated at 37°C for 15 minutes. Absorbance of the sample a sample and standard a standard against the blank was read. The color of the reaction was stable for at least 8 hours.

## **Results and Discussion**

Staphylococcal pneumonia is a generally progressive process in all age groups. Two main forms are recognized:

primary pneumonia due to direct inoculation through the respiratory tract and secondary or metastatic haematogenesis lung infection due to bacteremic seeding to the lung during the course of septicemia associated with the other sites of infection.

In this study, 79 patients were diagnosed to have pneumonia caused by *Staphylococcus aureus*. The various serum chemistry concentrations for sodium, potassium, chloride, creatinine and urea were determined.

For the control, the values obtained for Urea, Creatinine, Sodium, Potassium and Chloride were 14mg/dl, 0.47mg/dl, 136mmol/l, 4.8mmol/l and 98mmol/l respectively.

In respect to changes that occur in serum electrolytes, the results of this study show that there was a significant reduction in serum concentration of sodium and chloride while serum values of potassium were significantly increased [19].

Some studies conducted attributed the changes occurring in serum electrolytes during the course of respiratory disease to the hyperpyrexia in the acute course of the disease and metastatic infection of liver and kidneys resulting in hepatic and renal dysfunction [20].

High serum potassium levels usually occur in respiratory diseases especially if acidosis is present because H<sup>+</sup> ions accumulated in the extracellular fluids (ECF) is exchanging with potassium in the intracellular (ICF) leading to hyperkalemia [21].

From the result above, it can be inferred that the level of potassium was relatively high (Hyperkalemia) compared to the normal range of 3.5 to 5.0. Hyperkalemia is serum potassium >5.5 milliequivalents per liter, hence any level greater than 6mEq can be life-threatening, depending on the clinical setting [22,23]. Normal blood levels of potassium are critical for maintaining normal heart electrical rhythm. High blood potassium levels can lead to abnormal heart rhythm. Another important effect of hyperkalemia is interference with functioning of the skeletal muscle [24]. Central Hospital Ughelli had the highest mean value for potassium (8.02+0.779), CO for Urea, Yen for creatinine, Central Hospital Benin for sodium and Central Hospital Sapele for Chloride. For urea, pneumonia induced by *staphylococcus aureus* had no significant effect. This may be due to the level of infection or virulence of the organism on the pneumonia patient. Sodium levels drastically reduced (Hyponatremia) with the exception of SAP41, SAP83 and CB4. Increased sodium in the blood occurs whenever there is excess sodium in relation to water. Hyponatremia could be caused by kidney disease, too little water intake and loss of water due to diarrhea and

vomiting. Usually patients with pneumonia are at high risk of experiencing low sodium levels since the principal organ of effect is the lung with continuous production of cough with sputum. Pneumonia also affects the gastrointestinal tract

(GIT) causing nausea, vomiting and diarrhea. Due to loss of fluids, it is worthy to note here that the removal of sodium is faster than that of water from the body.

S/N	Names of Hospitals	Clinical Chemistry Parameters				
		Urea	Creatinine	Sodium	Potassium	Chloride
1	Central Hospital Asaba (ASB)	10.733 ± 0.689	0.597 + 0.102	133.666 ± 3.502	5.466 ± 1.422	96.333 ± 2.251
2	Central Hospital Sapele (SAP)	10.829 ± 0.859	0.486 + 0.038	143.428 ± 8.283	5.9857 + 0.6039	104.142 + 8.4936
3	University of Benin Teaching Hospital (UBT)	14.444 ± 2.404	0.489 + 0.033	136.111 + 3.140	5.0333 + 0.239	96.888 + 3.620
4	OT	10.143 + 0.690	0.571 + 0.076	134.571 + 2.439	5.657 + 1.028	97.143 + 1.345
5	Delta State University Teaching Hospital Oghara (OGH)	14.00 + 1.852	0.5 + 0	137.625 + 2.774	4.4125 + 0.264	96.625 + 2.875
6	Central Hospital Benin (CB)	10.40 + 1.140	0.57 + 0.067	143.8 + 6.906	6.02 + 0.7328	96.2 + 2.489
7	Central Hospital Warri (CW)	12.50 + 1.732	0.475 + 0.05	136.75 + 3.685	4.95 + 0.129	99.5 + 1
8	Central Hospital Ughelli (CU)	9.80 + 0.837	0.54 + 0.0547	133.4 + 3.847	8.02 + 0.779	94.8 + 2.683
9	Central Hospital Agbor (CA)	10.25 + 0.957	0.5 + 0	131.75 + 2.629	7.775 + 0.262	93.75 + 3.5
10	CS	9.75 + 0.500	0.55 + 0.057	133.75 + 1.707	5.45 + 0.685	96.75 + 2.629
11	Central Hospital Yenagoa (CY)	10.50 + 1.118	0.56 + 0.089	133.6 + 4.615	5.78 + 1.265	95.2 + 2.280
12	CO	15.00 + 1.445	1.5 + 1.671	139.25 + 6.5	22.35 + 31.118	97.25 + 1.258
13	YEN	10.40 + 0.894	1.7 + 2.404	135 + 4.183	5.76 + 1.167	97 + 2.738
14	Central Hospital Sagbama (CSA)	10.333 + 0.577	0.533 + 0.0577	137 + 1	7.866 + 0.231	96 + 1
15	Stella Obasanjo Women And Children Hospital Benin (SOH)	10.333 + 1.155	0.5 + 0	135.33 ± 4.163	6.1333 + 0.321	97 + 2.645

**Table 1:** Clinical Chemistry Parameters.

## Conclusion

This study is based on the ability of *Staphylococcus aureus* isolated from pneumonia patients to cause changes in the clinical chemistry parameters of the body. These physiological changes in potassium, urea, sodium, creatinine, and chloride were determined using spectrophotometric methods. It can be concluded that the *staphylococcus aureus* isolated from clinically diagnosed pneumonia patients was capable of altering the body's electrolyte level causing increase in potassium and decreased sodium and chloride

levels. There were slight changes in the urea level with a remarkable effect on the creatinine level.

## Conflict of Interest

No conflict of interest.

## Acknowledgment

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