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Microbiological Assessment of Quality Control in Governmental Laboratories in Khartoum State2016

تقويم مايكروبايولوجي لضبط الجودة في المعامل الحكومية بولاية الخرطوم .2016

A thesis Submitted for Partial Fulfillment for the Requirements of Master Degree in Medical Laboratory Science (Microbiology)

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الآية

بسم الله الرحمن الرحيم

قال تعالي :-

(وَتَرَى الْجِبَالَ تَحْسَبُهَا جَامِدَةً وَهِيَ تَمُرُّ مَرَّ السَّحَابِ صُنْعَ اللَّهِ الَّذِي أَتْقَنَ كُلَّ شَيْءٍ إِنَّهُ خَبِيرٌ بِمَا تَفْعَلُونَ ﴿ 88 ﴾)

صدق الله العظيم

(النمل : 88)

عن هشام بن عروة عن أبيه، عن عائشة رضي الله عنها قالت :قال رسول الله صلى الله عليه وسلم: (إن الله يحب إذا عمل أحدكم عملا أن يتقنه) أخرجه أبو يعلى والطبراني

DEDICATION

I DEDICATE THIS WORK:

TO WHOM STAND WITH ME WITHOUT WAITING .....

MY MOTHER FATIMA

TO THE SPIRIT OF MY MISSED .....

MY FATHER ELDERDEREE

TO MY BROTHERS AND SISTERS WHOM I LOVE AND APPRECIATE

TO MY WIFE

TO MY LOVELY DAUGHTERS

BASMALA , LENA & ASMAA

TO MY SON

MOHAMMED

TO MY FRIENDS

TO MY DEAR TEACHERS WHO HAVE GREAT CREDIT FOR WHAT I'M INTO RIGHT NOW

TO ALL WHO LOVED ME AND WISHED ME CONTINUED PROGRESS

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Abstract

Quality is an important issue in medical laboratories, it leads to right and trusted results, which will guide the doctor and patient to the right treatment without wasting time and money, and then it will lead to healthy individuals and communities. In this study, we assessed the quality measures in eleven microbiology laboratories in Khartoum State hospitals by applying the standard clauses of ISO 15189 and checking the reproducibility of these laboratories by using three NCTC/ATCC different organisms with different properties. The objective of this study was to assess the implementation of quality control standards in the governmental microbiology laboratories in Khartoum State.

This is non-interventional (Observational) Descriptive – cross sectional design study was conducted between January to March 2016. Three standard organisms(*S.aureus*NCTC12903/ATCCR27853),(*E.coliNCTC12241*/ATCCR25922),(*Ps.aeruginosa* NCTC12973/ATCC R 29213) distributed to check the reproducibility and sensitivity tests. ISO 15189:2007 checklist was used to assess the managerial and technical clauses applied inside these laboratories. SPSS and excel programs were used to analyze the data. The results introduced in tables.

The results showed that the percentage of applied clauses of ISO 15189 checklists was between 34% to 64%.The Equipment 54.4% were old (not automated).Laboratories personnel have 52.5% from the international standards. Reagents and Supplies have 66% .The pre-analytical was the main source of false results 55.7% while the analytical was having 65% and the post analytical was having 75%. Personnel 52.5% no continuous education program available for the staff, no procedure to control personnel performance and no records maintained for all the staff.

Equipment 54.4% Laboratories not furnished with all equipment required for services just it were finished with bacteriology equipments, Instruments had no documented maintenance, no verifications labels. The quality assurance applied partially 32.1%. Reproducibility: Twenty-four out of thirty-three were correct final identification answers 72.7%. There should be written strategies for antibiotic sensitivity tests. Assuring quality was 32.1% and continuous improvement was 30.4%, clauses were poorer. Documents 44% were not an important issue inside the laboratories.–Internal audit 67.5% is functionless and with no impact if was done because there were no adopted appropriate corrective and preventive actions.

The identification results received for the three standard organisms (NCTC/ ATCC) types; One hospital 9.1% failed to provide identification result for the three organisms because it was not having biochemical test sets. One result 3% was false microscopical result (Gram positive instead of Gram negative). Five results 15.2% had nomenclature error they wrote the genus without species. Nine final identification results 27.3% were false.

The antibiotics susceptibility tests results indicate that one laboratory 9.1% used multidisc for Gram negative and positive bacteria.

The study showed that: all these laboratories need to review their quality management system, policies, procedures, and processes to control the testing activities.

مستخلص الدراسة

الجودة في استخراج النتائج المعملية مهمة جداً وذلك لضمان صحة النتيجة المستخرجة والتي يترتب عليها علاج المرضي ولذا فقد أجريت هذه الدراسة في معامل الأحياء الدقيقة بالمستشفيات الحكومية بولاية الخرطوم في الفترة من يناير إلي مارس 2016 وقد كان الهدف من هذه الدراسة معرفة مدي تطبيق هذه المعامل للمعايير القياسية العالمية لنظم إدارة الجودة. وقد كانت الدراسة عبارة عن دراسة وصفيه مقطعيه دون تدخل وقد تم فيها وصف الوضع الراهن بهذه المستشفيات . أيضا تم مراجعة الجودة في استخراج النتائج لهذه المستشفيات وذلك بتوزيع ثلاثة عينات لبكتريا قياسيه بخصائص مختلفة وذلك لإعادة التعرف عليها بواسطة هذه المعامل ولإجراء فحص الحساسية ومن ثم المقارنة بين نتائج المستشفيات والنتيجة الصحيحة .

تم استخدام قائمة مواصفة الجودة ISO 15189 لمتابعة ومعرفة مدي تطبيق المعايير القياسية للجودة وكذلك تم استخدام بكتريا قياسيه لمعرفة دقة الفحوصات المعملية في التعرف علي البكتريا واختبار الحساسية للبكتريا .

إن نتائج الدراسة قد أظهرت إن متوسط تطبيق معايير الجودة العالمية للمعامل ISO 15189 تتراوح بين 34% إلي 64% , وحسب تحليل الوضع الراهن لهذه المعامل فهي ضعيفة في كثير من جوانب الجودة الشاملة, كما إن العاملين بهذه المعامل قد حصلوا علي نسبة 52.5% من المعايير العالمية وذلك لأنه لا توجد خطط واضحة لإدارة العاملين كما انه لا يوجد وصف وظيفي مكتوب بهذه المعامل. الأصباغ والمستهلكات حصلت علي (66%): لا توجد طريقه مكتوبة للاختيار والشراء, كما إن المستهلكات غير موحدة المصدر لمعامل الولاية. الأخطاء التي تحدث قبل عملية تحليل العينة كانت هي المصدر الرئيسي للأخطاء في النتائج وقد حصلت على نسبة 55.4% كما انه لا توجد تعليمات مكتوبة لقبول أو رفض استلام العينات وفي ذات الأثناء فأن العمليات التي يجب أن تتم أثناء التحليل المعملي قد نفذت بنسبة 65% والعمليات التي يجب تتم بعد التحليل المعملي قد نفذت بنسبة 75%.نال العاملين بالمعامل نسبة 52.5% من المواصفات لعالميه وذلك لنقص في برنامج التدريب المستمر, لا يوجد نظام لمراقبة أداء العاملين كما انه لا توجد سجلات للعاملين. نالت المعدات داخل المعمل 54.4% وذلك لأن المعامل ليست بها كل المعدات التي تحتاجها كمعامل للأحياء الدقيقة ويوجد فقط معدات للفحوصات البكتيرية , الاجهزه ليس لها سجلات صيانة ولا علامات للتحقق الأولي من عملها.عمليات ضمان الجودة نفذت بنسبة 32.1% فقط . إعادة الحصول علي النتائج الصحيحة كان بنسبة 72.7%. يجب وضع سياسات مكتوبة لاختيار المضادات الحيوية لكل

معامل الولاية . ضمان الجودة 32.1% والتحسين المستمر30.4% كانت هي اضعف الحلقات . التوثيق داخل المعمل 44% ليس له الاعتبار الذي يستحقه كما انه لا يوجد طريقه مثلى لحفظ السجلات . الإشراف الداخلي غير فعال وذلك لأنه لا توجد إجراءات تصحيحيه ووقائية متبناة .

تم استلام نتائج التعرف والحساسية للبكتريا القياسية التي تم توزيعها على المستشفيات وكانت النتيجة كما يلي : هنالك مستشفي واحد 9.1% لم يتعرف علي أي بكتريا وذلك لأنه لا يملك المحاليل التي تمكنه من إجراء الفحوصات الكيميائية للتعرف علي البكتريا. هنالك نتيجة واحده 3% للفحص المهجري خاطئة حيث كتبت النتيجة موجبة القرام ولكن البكتريا سالبة القرام. هنالك خمس نتائج 15.2% به خطأ في كتابة الاسم العلمي حيث كتب اسم الجنس دون كتابة النوع. هنالك تسع نتائج 27.3% للتعرف النهائي للبكتريا كانت خاطئة .

نتيجة اختبار الحساسية أظهرت أن هنالك معمل بيستخدم أقراص المضادات الحيوية التي تستخدم كحزمه للبكتريا الموجبة القرام والسالبة القرام دون مراعاة لنوع ومكان الالتهاب البكتيري.

هذه الدراسة أظهرت أن كل معامل الأحياء ألدقيقه بولاية الخرطوم تحتاج لمراجعة نظم إدارة الجودة , السياسات , الطرق والعمليات وذلك حتى يتم ضبط النتائج بهذه المعامل وضمان صحتها.

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Abbreviations

1	ATCC	American Type Culture Collection
2	BCR	Bureau Cmmunautairede reference
3	CAP	College of American Pathologists
4	CLIA	Clinical Laboratory improvements Amendment
5	CLSI	Clinical and Laboratory Standards Institute
6	CLMA	Clinical Laboratory Management Association
7	COLA	Commission Office of Laboratory Accreditation
8	CV	CO-Efficient of Variation
9	EQA	External Quality ASSUARNCE
10	EQAS	

External Quality Assessment scheme	EQC	External Quality Control
12	HCFA	Health care financing administration
13	IQC	Internal Quality Control
14	ILAC	International Laboratory Accreditation Cooperation
15	ISO	International standards organization
16	JCAHO	Joint Commission Accreditation of Health Organization
17	MIC	Minimum Inhibitory Concentration
18	NCCL	National Committee for Clinical and Laboratory Standards
19	NCTC	National collection of type culture
20	NIST	National Institute for Standards and Technology
21	PT	Proficiency testing
22	QC	Quality Control
23	SDI	Standard Deviation Index
24	PCR	Polymerase Chain Reaction
25	QMS	Quality Management System
26	STD	Standard
27	bp	Base pair

1. INTRODUCTION

1.1 Introduction

In Khartoum State there is 25 governmental hospital, in fact only 15 governmental hospital that provide microbiology services and there no previous study done to evaluate these laboratory performance. These laboratory dose not used perfectly by the physicians and the patients and that is for unknown reasons and this will cost money and contribute to antibiotic resistance, on the other hand there is no unique quality management system (national) adopted to manage the quality of the microbiological services provided.

By the way the microbiology laboratories does not provide a full microbiology services, it just provide bacteriology services not used even that properly, although the infectious disease are the major causes of morbidity and mortality.

Actually, the most requests are for stool and urine culture, there are rare or no request for chest infection, CSF, synovial fluid. The role of microbiology laboratories in community health is partially absent.

Quality controlprocedures are critical to maintain and improve the accuracy, precision and reliability of the data produced in any laboratory analysis. These should be implemented in each laboratory to ensure that appropriate sampling and analytical procedures are followed, laboratory and field equipment are regularly checked and calibrated, and staff are adequately trained and supervised. QC checks are what a Laboratory does to ensure that its quality assurance program is working (Hassan *et al*., 2008)

Microbiology laboratories shall use quality control procedures to ensure the accuracy, reliability and reproducibility of the various tests used in the isolation, identification and antimicrobial susceptibility testing of

microorganisms, and in the performance of serological testing. The extent of quality control testing done will be determined by the scope of clinical testing performed in each laboratory (College of Physician & Surgeon Of Saskatchewan, 2010).

1.2 Rationale

Unfortunately, thereare no enough implementation of quality, and most medical laboratory workers are not aware enough about quality and safety precautions. They do not take quality as priority although it is important issue in medical laboratories practice. Wrong laboratory results will lead to misdiagnosis and of course wrong treatment, which absolutely lead to bad prognosis. This actually will affect the patients' health and have impact on public health and economy. This study designed to detect the malfunctions, bad habits and wrong concept about quality. There are no studies concern in this topic , to the best of my knowledge, in Sudan.

1.3 The study objectives

1.3.1 General Objective

Microbiological assessment of quality control in governmental hospitals laboratories in Khartoum State.

1.3.2 Specific Objectives

- To measure the efficiency of test results.
-To check the instrument calibration and maintenance programs.
- To check the sensitivity test results.
- To compare between pre-analytical, analytical and post-analytical source of false results.

2 Literature Review

2.1 Defining Quality

Quality means developing statements regarding the input, processes and outcome standards that the health care delivery system must meet for its population in order to achieve optimum health gains (National Pathology Accreditation Advisory Council, 2001).

The general concept of quality means the measure of excellence orstate of being free from defects, deficiencies and significant variations (Alneil, 2011).

In health care, quality means doing the right thing, at the right time, in the right way, for the right person and having the best possible results. This means that every health planers, doctors, hospitals, and other health providers must give high quality care.

Quality is defined as the degree to which a product or service meets or exceeds a customer's requirements and expectations.

Quality is the result of several ongoing processes. It requires many individuals performing appropriate activities at the correct time during the plan development process. Quality control does not just consist of a review after a work product is completed (Haas, 2013).

2.2 Defining Quality Control (QC)

The final inspection and testing of the finished product to ensure it is compliance with predetermined performance criteria (Traynor, 2012).

QC procedures are critical to maintain and improve the accuracy, precision and reliability of the data produced in any laboratory analysis. These should be implemented in each laboratory to ensure that appropriate sampling and analytical procedures are followed, laboratory and field equipment are regularly checked and calibrated, and staff are adequately trained and supervised. QC checks are what a Laboratory does

to ensure that its quality assurance program is working (Hasan *et al*.,2008).

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Quality Control on the other hand includes those activities that are undertaken to confirm that test and measurements results are accurate and reliable. These activities include, but are not limited to participation in proficiency tests and other inter-laboratory comparisons, regular use of certified standard reference materials, secondary or sub-reference materials, in-house reference standards, testing or measurement of multiple samples (duplicates or replicates) (Hasan *et al*.,2008).

2.2.1 Internal and External

Random and systematic errors must be detected at an early stage and then every effort should be taken in order to minimize them. The strategy for their detection consists of specific quality control methods which are divided in two categories:

2.2.1.1. Internal Quality Control (IQC)

It includes all QC methods, which are performed every day by the laboratory personnel with the laboratory's materials and equipment. It checks primarily the precision (repeatability or reproducibility) of the method (Karkalousos and Evangelopoulos .,2011).

2.2.1.2 External Quality Control (EQC)

It includes all QC methods, which are performed periodically (i.e. every month, every two months, and twice a year) by the laboratory personnel with the contribution of an external center (referral laboratory, scientific associations, diagnostic industry etc.). It checks primarily the accuracy of the laboratory's analytical methods. However, there are certain EQC schemes that check both the accuracy and the precision. Other terms for external quality control are: inter-laboratory comparisons, proficiency testing (Karkalousosand Evangelopoulos .,2011).

2.2.2 Quality Control Plan

Is a comprehensive, well-defined, written set of procedures and activities aimed to delivering products that meet or exceed a customer's expectations, as expressed in contract documents and other published sources. A quality control plan will identify the organization or individuals responsible for quality control and the specific procedures used to ensure delivery of a quality product. A quality control plan will also detail quality assurance measures and the method of accountability and required documentation (Haas, 2013).

2.2.3 Metrics of Internal and External Quality Assessments Schemes

The metrics of internal and external quality control are based on statistical science (e.g. SDI, CV, Z-score) and they are graphically represented by statistical charts (control charts). Some of them are common in other industries while others specific for internal or external quality control in clinical laboratories (Karkalousosand Evangelopoulos .,2011).

2.3 Defining Quality Assurance(QA)

Quality assurance is a systematic and planned approach to assessing, monitoring and improving the quality of health services on a continuous

basis. It promotes confidence, improves communications and allows clearer understanding of community needs and expectations.

. Quality assurance is oriented towards meeting the needs and expectations of the patient and the community. Quality Assurance focuses on the way we work, our activities, and processes of health care delivery. Quality assurance employs the use of data to analyze how we are working and delivering health services. Quality Assurance involves a multi-disciplinary team approach to problem solving and quality improvement.

In practice Quality Assurance is a continuous process and the quality assurance cycle can be used to guide your activities.

High quality health services do not mean luxury or "high-tech" services. As a health care provider giving attention to quality services is very essential for us whatever our resource maybe.

A lot of QA change can occur without excess additional resources.

Everyone is responsible for quality, from National down to individual level (Quality Assurance & Standardization Division Ministry Of Health Thimphu Bhutan, 2007).

Quality assurance includes all the activities undertaken by a laboratory to ensure that reliable and accurate testing or measurement will be undertaken at all times. These activities include document control, laboratory internal audits, management review, sampling, handling and storage of samples, control of non-conforming work, complaints and corrective action procedures, technical and quality records (Hasan *et al*.,2008).

2.3.1 Medical Laboratory Process

• Specimen collection (Pre-analytical)

• Specimen transport (Pre-analytical)

• Specimen receipt (Pre-analytical)

• Specimen processing (Pre-analytical)

• Testing (analytical)

• Interpretation (analytical)

• Reporting (Post-analytical) (Poutanen, 2010)

2.4 Quality Assurance Programs

Are efficient ways of maintaining the standards of performance of diagnostic laboratories, and of upgrading those standards where necessary. In microbiology, quality goes beyond technical perfection to take into account the speed, cost, and usefulness or clinical relevance of the test. Laboratory tests in general are expensive and, with progress in medicine, they tend to use up an increasing proportion of the health budget (Vandpitte, 2003).

Quality assurance is the sum of all those activities in which the laboratory is engaged to ensure that test results are of good quality. It must be:

— Comprehensive: to cover every step in the cycle from collecting the specimen to sending the final report to the doctor.

— Rational: to concentrate on the most critical steps in the cycle;

— Regular: to provide continuous monitoring of test procedures;

—Frequent: to detect and correct errors as they occur(Vandpitte, 2003).

Quality assurance (QA) in health laboratories incorporates all the factors that may influence the generation of reliable results. It comprises two key components. Internal quality control (IQC) includes appropriate measures taken during day-to-day activities to control all possible variables that can influence the outcome of laboratory results. This is a continuous process that operated concurrently with analysis. External quality assessment scheme (EQAS) is the other component. This component is necessary to ensure comparability of results among laboratories (Kumari and Sharma.,2005).

2.4.1 Proficiency Testing

Proficiency testing had been shown to be a valuable tool for recognizing deficient laboratory performance long before passage of CLIA 67. In the mid-1940s, Sunderman and others operating clinical laboratories in Philadelphia, Pa., became concerned over incidents in which physicians had divided samples of blood and obtained substantially different results from different laboratories. Under the auspices of the Philadelphia County Medical Society, the first proficiency testing program was initiated. This program was so revealing of inadequacies that it became the impetus for organization of the CAP in 1946. The first microbiology survey was conducted by the CAP in 1959 and involved 600 laboratories. Following CLIA 67, and later with passage of the Medicare Act and creation of the HCFA, the role of these organizations in conducting proficiency testing was expanded (Bartlett*et al*., 1994).

2.5 Quality Management

Are all activities of the overall management function that determine quality policy, objectives, and responsibilities, and implement them by means such as quality planning, quality assurance, quality control, and quality improvement within the system (Haas, 2013).

Always remember that: Success or failure of a quality system is dependent on the laboratory staff's:

Knowledge, skills, motivation and Commitment (Poopak, 2013)

2.6 Factors that affect the reliability and reproducibility of laboratory results

Sources of error may include the following:

• Personnel. The performance of the laboratory worker or technician is directly related to the quality of education and training received the person's experience, and the conditions of employment.

• Environmental factors. Inadequate working space, lighting, or ventilation, extreme temperatures, excessive noise levels, or unsafe working conditions may affect results.

• Specimens. The method and time of sampling and the source of the specimen are often outside the direct control of the laboratory, but have a direct bearing on the ability of the laboratory to achieve reliable results. Other factors that the laboratory can control and affect quality are the transport, identification, storage, and preparation (processing) of specimens.

The laboratory therefore has a role in educating those taking and transporting specimens. Written instructions should be made available and regularly reviewed with the clinical and nursing staff.

• Laboratory materials. The quality of reagents, chemicals, glassware, stains, culture media, and laboratory animals all influence the reliability of test results.

• Test method. Some methods are more reliable than others.

• Equipment. Lack of equipment or the use of substandard or poorly maintained instruments will give unreliable results.

• Examination and reading. Hurried reading of results, or failure to examine a sufficient number of microscope fields, can cause errors.

• Reporting. Transcription errors, or incomplete reports, cause problems (Vandepitte, 2003).

2.7 Quality Criteria in Microbiology

Clinical microbiology plays a crucial role in the health of individual persons and the communities in which they reside. Most microbes that live on or within the body are beneficial and help keep individuals healthy. Distinguishing between microorganisms that are beneficial and those that are disease producing is a critical function of the clinical microbiologist (Miller *et al*.,2008).

Clinical microbiology is mostly based on interpretation while giving the results. There may be laboratory errors, which can lead to problems in the process. Such errors may occur in the pre-analytical, analytical, and post-analytical steps and influence the therapy of the patient, in addition to misleading the clinician (Kusum and Silva.,2005).

All laboratories performing microbiology shall have appropriate internal quality control procedures using CLSI guidelines for antibiotic susceptibility testing and quality control of media. The most recent update of the following CLSI documents should be followed:

Quality Assurance for Commercially Prepared Microbiological Culture Media.

Performance Standards for Antimicrobial Disk Susceptibility Tests

ATCC organisms shall be used for proper quality control of media and of antimicrobial susceptibility testing (College of Physician & Surgeon Of Saskatchewan.,2010).

2.7.1 Clinical Relevance

An important criterion of quality for a microbiological test is how much it contributes to the prevention or cure of infectious diseases; this is called its clinical relevance. Clinical relevance can only be ensured when there is good communication between the clinician and the laboratory.

2.7.2 Reliability

For tests that give quantitative results, reliability is measured by how close the results are to the true value.

2.7.3 Reproducibility

Two things reduce the reproducibility or precision of a microbiological test:

1. Lack of homogeneity. A single sample from a patient may contain more than one organism. Repeat culturing may therefore isolate different organisms.

2. Lack of stability. As time passes, the microorganisms in a specimen multiply or die at different rates. Repeat culturing may therefore isolate different organisms. To improve precision, therefore, specimens should be tested as soon as possible after collection.

2.7.4 Efficiency

The efficiency of a microbiological test is its ability to give the correct diagnosis of a pathogen or a pathological condition (Vandepitte,2003)

The laboratory should have documented policies and procedures to implement corrective actions when nonconformance is detected.

Corrective actions are not only associated with failures in the quality of test results, although this is an obvious laboratory outcome for supervisors to act upon. Corrective actions may also be required when problems in the quality system are identified following reviews, audits, complaints or other events affecting laboratory function are observed or recorded (National Pathology Accreditation Advisory Council .,2001).

2.7.5 Verification and Validation

Verification is the first step, one-time confirmation that the test works as indicated by manufacturer

Validation is the continual confirmation that the test still works in the laboratory setting after verification requirements have been satisfied (Fuller .,2005).

2.8 Antibiotic Susceptibility Testing

Bacteria demonstrate two kinds of resistance to antibiotics, namely intrinsic resistance and acquired resistance. Intrinsic resistance means that the species was resistant to an antibiotic even before its introduction. Acquired resistance means that the species was originally susceptible to an antibiotic, but later became resistant. Bacteria can acquire antibiotic resistance either by mutation or through exchange of genetic material among same or closely related species. The sudden acquisition of

resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is even more significant problem. It is because of the acquired resistance that bacterial isolates must be subjected to antibiotic susceptibility testing. A bacterium showing reduced susceptibility or resistance to an antibiotic implies that it should not be used on the patient (Sirdharroa,2014).

As the "gold standard" of the antibiotic susceptibility of an organism is the minimum inhibitory concentration (MIC) of the antibiotic under test all the methods of susceptibility testing must relate to this value. Moreover, the MIC must be determined by an internationally standardized technique. The agreed gold standard test is the agar dilution technique originally proposed by Ericsson and Sherris in 1971. (Bell *et al*.,2009).

Susceptibility testing:

1) Agar diffusion with disk
2) Agar diffusion with E-test
3) MIC-determination using Agar dilution method.

MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the organism. The principle is simple: Agar plates, tubes or microtitre trays with two-fold dilutions of antibiotics are inoculated with standardized inoculums of the bacteria and incubated under standardized conditions following NCCLS guidelines. The next day, the MIC is recorded as the lowest concentration of antimicrobial agent with no visible growth (Henderksen,2003).

2.9 International Standard and Accreditation Bodies

In laboratory medicine, efforts to develop guidelines, standards, policies, and best practice recommendations have typically been independent ventures that serve specific fields or professions. Professional organizations and industry associations, such as the College

of American Pathologists (CAP), the Clinical and Laboratory Standards Institute (CLSI), and the Clinical Laboratory Management Association (CLMA), have developed approaches to recommending and disseminating quality practices. In some cases, government agencies and accrediting bodies have recognized these recommendations as meeting regulatory and accreditation requirements (Snyder *et al*.,2007).

There are worldwide different organizations delivering:

- Standardized reference method (written standards)
- Certified reference material (primary standards)
- Certified reference material (matrixed samples with certified target values).

There are written standards edited by national institute for standards and technology (NIST) in USA, by the National Committee for Clinical and Laboratory Standards (NCCL, USA) and in Europe by the Bureau Cmmunautairede reference (BCR). (NIST) and (BCR) distribute primary standards and certified reference materials (Renauer, 1995).

ISO 15189 Medical laboratories – Particular requirements for quality and competence is a standard that contains the requirements necessary for diagnostic medical laboratories to demonstrate their competence to deliver reliable services.

The scope of ISO 15189states the standard is for "use by medical laboratories in developing their quality management systems and assessing their own competence and for use by accreditation bodies in confirming or recognizing the competence of medical laboratories". The introduction States: "If a laboratory seeks accreditation, it should select an accrediting body which operates to appropriate international standards and which takes into account the particular requirements of medical laboratories". Therefore, laboratories that meet its management and technical requirements qualify for recognition by accreditation bodies that

are members of the International Laboratory Accreditation Cooperation (ILAC). Clinical personnel responsible for patient care can be confident that medical laboratories accredited to ISO 15189 are competent to produce timely and reliable diagnostic examination results (Working Group, 2012).

Accreditation processes are widely known in clinical laboratories and medical services. In United States of America (USA): Collage of American pathologist (CAP), Commission Office of Laboratory Accreditation (COLA), and Joint Commission Accreditation of Health Organization (JCAHO) have an established know-how in the field. However, the ISO 15189 standard. since its publication in 2003, is gaining more and more acceptance by accreditation bodies worldwide as the standard for medical laboratories and has been adopted as the accreditation criteria used by many countries, including New Zealand, Canada, Hong Kong, Thailand, while others including Malaysia, China, Japan, are also planning to start accreditation of laboratories using this new standard(Alniel,2011).

For accreditation, the rules require that the accreditation process be carried out by third party organization: that means not by peers, not by first party (suppliers) and not by second party (customers). third party is defined a person or body that is recognized as being independent of the parties involved, in this case independent of the laboratory or the laboratory parent organization (Alniel,2011).

2.10 Standard Organisms

Three different standard organisms were used (NCTC/ATCC) types.*S. aureus* (NCTC12903/ATCC R 27853, LOT 10/21, strain sourced from NCTC.*E.coli* (NCTC12241/ATCC R 25922, Strain sourced from NCTC. LOT 02/35.*Ps.aeruginosa* (NCTC12973/ATCC R 29213 , LOT 14/55 strain sourced from NCTC.(Appendix I)

2.11ISO 15189-check list :-

ISO 15189-check list usually use as an audit tool to evaluate the whole lab (setup, performance, instrumentation, QC programmes, correction actions, responsibilities, documentations, etc.).(Appendix III)

2.12 Biochemical Test

2.12.1. Catalase test

To differentiate Staphylococci (catalse positive) from Streptococci (catalase test negative).

2.12.2. Citrate utilization test

To differentiate members of Enterobacteriaceae family.

2.12.3. Coagulase test

To identify *Staphylococcus aureus*. Coagulase test differentiates *Staphylococcus aureus* (positive) from coagulase negative staphylococci (CONS), such as *S.epidermidis*, *S. saprophyticus*.

2.12.4. DNase test

This test is used to determine the ability of an organism to hydrolyze DNA. It is primarily used to identify *Staphylococcus aureus*

2.12.5. Indole test

This test is used to determine the ability of an organism to split tryptophan to form the compound indole. It is used differentiate gram negative rods particularly *E. coli* in microbiology laboratory.

2.12.6. Oxidase test

To help identify *Neisseria, Pasteurella*, *Vibrio*, *and Pseudomonas*. This test is used to determine the presence of bacterial cytochrome oxidase.

2.12.7. Urease test

Urease test is used to determine the ability of an organism to produce the enzyme urease which hydrolyzes urea. This test is done to help identify *Proteus*, *Morganella*, *Yersinia. Enterocolitica, Helicobacter pylori* .

2.13 Antibiotics Susceptibility Testing(SensitivityTesting)

Kirby-Bauer antibiotic testing (KB-testing or disk diffusion antibiotic sensitivity testing): is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. Known quantities of bacteria are grown on agar plates in the presence of thin wafers containing relevant antibiotics. If the bacteria are susceptible to particular antibiotics, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition) ,(Sirdharroa,2014).

The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. These along with the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacterium. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection,(Sirdharroa,2014).

All aspects of the Kirby-Bauer procedure are standardized to ensure consistent and accurate results. Because of this, a laboratory must adhere to these standards. The media used in Kirby-Bauer testing must be Mueller-Hinton agar at only 4 mm deep, poured into either 100m or 150mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4.

Inoculation is made with a broth culture diluted to match a 0.5 McFarland turbidity standard, which is roughly equivalent to 150 million cells per mL.(Sirdharroa,2014)

3. Materials and Methods

3.1 Study Design

It was non-interventional (observational) descriptive – cross sectional design.

3.2 Study Area

The study was done in Khartoum State at eleven governmental laboratories.

3.3 Study Duration

The study was conducted during the period from January to March 2016.

3.4 Inclusion and Exclusion Criteria

Laboratories of governmental hospitals with microbiology department were included in this study.

Laboratories of non-governmental hospitals without microbiology departmentwere excluded in this study.

3.5 Sample Size

Three standard isolate samples were distributed to eleven governmental hospitals laboratoriesin :Bahri Renal Centre , Alnaw, Jaafar ibn auf, Ibn seena, Bashaier, Alturky, Bahry, Haj alsafi, Ahmed Gasim, Atfal Omdurman, and Albuluk.

3.6 Ethical Consideration

Verbal consent was taken from all hospitals after full explanation of the purpose of the research.

3.7 Data processing

3.7.1 StandardSamples (NCTC/ATCC)

Three Samples with three different standard organisms were chosen to evaluate theefficiency of laboratories, these organisms were with different morphological, biochemical and drug sensitivity properties, and they were NCTC/ATCC types.

They were subcultured in nutrient agar, distributed and tested with these laboratories for staining properties, identification, and sensitivity testing. Three samples for each laboratory, and these samples were labelled by one, two and three as follow:

- One for Gram positive organism which was *S. aureus* (NCTC12903/ATCC R 27853, LOT 10/21, strain sourced from NCTC.
- Two for Gram negative organism, which was *E.coli* (NCTC12241/ATCC R 25922, Strain sourced from NCTC. LOT 02/35
- Three for Gram negative organism, which was *Ps.aeruginosa* (NCTC12973/ATCC R 29213 , LOT 14/55 strain sourced from NCTC.

3.7.2 Recheck of Standard Samples

All standard organisms were tested for

3.7.2.1 Viability of the Organisms

Viability of the Organisms were determine using deferential media MacConkeyAgar,Manitol Salt Agar(MSA) for *S.aureus* and Eosin Methylene Blue(EMB) for *E.coli* and testedfor antibiotics susceptibility testing.The three organisms weresuccessfully passed all tests in Laboratories Management.

3.7.2.2 Molecular Techniques

Molecular techniques were used to confirm the type of three different bacterial organisms as the follow:

3.7.2.2.1 DNA Extraction

1. Two to three colonies of a fresh overnight culture of bacterial cells was suspended by a loop full in 100 µl lysis.
2. Buffer (InstaGene Matrix, Biorad®) in 1.5 ml Eppendorf tube, vortexed for 15 sec and incubated at 56°C for 1 hour.
3. Mix well by vortexing and incubated at 95°C for 1 hour.
4. Mix well by vortexing and centrifuged at 13200 rpm for 5min.
5. Store DNA samples at -20°C.

* Vortex and centrifuge the DNA suspension (13200 rpm for 5 min), before use .

3.7.2.2.2 Primer Design

S.aureus

Forward 5'-GCGATTGATGGTGATACGGTI-3'

Reverse 5'-AGCCAAGCCTTGACGAACTAAAGC-3'

E. coli

Forward 5'GGGTGAAGTAAGTGACCAGAATCA3'

Reverse :5'-CACGT CAATGGGACGATGTC-3'.

Pseudomonas aeruginosa

Forward 5′- ATGAACAACGTTCTGAAATTCTCT -3′

Reverse 5′- CTTGCGGCTGGCTTTTTCCAG -3′

3.7.2.2.3PCR Protocol

1. In 200 ul of eppendoff tube 5.4ul from master mix were added.
2. Distal water(14.1 ul) were added
3. 0.5 ul from primer were added.
4. The samples were run in thermocycler(Multi gene opti max).
5. denaturation was done at 94°C for 1 min
6. Annealing was done at 55°C for 0.5 min
7. DNA extension was done at 72°C for 1.5 min,the samples were run for 30 cycles .
8. Final cycle, the reaction was terminated by keeping it at 72°C for 3.5 min.
9. The PCR products were stored in the cycler at 4°C until used.

3.7.2.2.4Gel Electrophoresis

The amplified products were separated on 2% agarose and ethidium bromide staining, a 267bp fragment wasobtained for *S.aureus*, 249bp for*Pseudomonas aeruginosa*and 259 bpfor *E.coli*.

The PCR product was run on gel electrophoresis from(Cleaver scientific CS_300V) and mini gel tank.

The gel was visulised using gel documentation system (Cleaver scientific LTD micro doc).

3.7.3 ISO 15189-Check List

ISO 15189-check listwas used as an audit tool to evaluate the whole lab (setup, performance, instrumentation, QC programmes, correction actions, responsibilities, documentations, etc.)

3.8 Data Collection Technique

The data was collected by

1- Direct interviews with staff members were done according to adopted module of ISO 15189, which is specific for medical laboratory standards, this module compose of 23 requirements (managerial and technical) these requirements were designed in checklist with three options: yes, no and partial ,Yes has two marks, No with zero mark and Partial with one mark.

2-Laboratory results (identification and sensitivity test) for the standard organisms were observed after 3 days.

3.9 Methodology

3.9.1 Organisms Identification

- Morphological identification (colonial morphology, cell shape and cell size).
- Differential staining (Gram staining).

3.9.1.1BiochemicalTests

I Catalase Test: To differentiate Staphylococci (catalse positive) from Streptococci (catalase test negative).

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24hours old.

II CoagulaseTest: To identify *Staphylococcus aureus*. Coagulase test differentiates *Staphylococcus aureus* (positive) from coagulase negative staphylococci (CONS), such as *S.epidermidis*, *S. saprophyticus*.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin .

III DNaseTest: This test was used to determine the ability of an organism to hydrolyze DNA. It is primarily used to identify *Staphylococcus aureus* .

Principle

Deoxyribonucleasehydrolyzes deoxyribonucleic acid (DNA).The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are testedfor DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNA-ase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis.

IV Citrate Utilization Test

To differentiate members of Enterobacteriaceae family.

Principle

This test is one of several techniques used occasionally to assist in the identification of enterobacteria.The test is based on the ability of an organism to use citrate as its only source of carbon.

V Indole Test: This test was used to determine the ability of an organism to split tryptophan to form the compound indole. It was used differentiate Gram negative rods particularly *E. coli* in microbiology laboratory.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl amino benzaldehyde. This reacts with the indole to produce a red coloured compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

VI Oxidase Test: To help identify *Neisseria*, *Pasteurella*, *Vibrio*, *and Pseudomonas*. This test was used to determine the presence of bacterial cytochrome oxidase.

Principle

A piece of filter paper is soaked with a few drops of oxidasereagent. A colony of the test organism is then smeared on the paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

VII Urease Test: Urease test was used to determine the ability of an organism to produce the enzyme urease which hydrolyzes urea. This test is done to help identify *Proteus*, *Morganella*, *Yersinia. Enterocolitica, Helicobacter pylori*.

Principle

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as

shown by a changing colour of the indicator to pink-red. (Cheesbrough, 2006).

3.9.1.2*S.aureus*Identification

Firstly, Gram stain was made, *S.aureus* was Gram +ve cocci then catalase test was made, *S.aureus* was catalase +ve.

Blood agar

S.aureus produces yellow to cream or occasionally white 1–2 mm in diametercolonies after overnight incubation. Pigment was less pronounced in young colonies. Some strains are beta hemolytic when grown aerobically, Colonies were slightly raised and easily emulsified.

MacConkey agar

Smaller (0.1–0.5 mm) colonies were produced after overnight incubation at 35–37 ºC. Most isolates were lactose fermenting.

- DNase activity was tested on DNase test agar following the manufacturer's recommendations (Difco Laboratories, Detroit, Mich.). Only strong DNase activities (clearing zone around growth) were recorded as positive.
- The coagulase test was performed with plasma following the recommendations of the manufacturer (Bio-Merieux). Results were recorded after 4 and 24 h of incubation at 37°C. Weak coagulase activities were recorded as positive.

3.9.1.3*E.coli* Identification

- Firstly, gram stain was made, they were Gram negative rods. Then they did oxidase test, *E.coli* was oxidase negative then they did biochemical tests. The results of *E.coli* were as follow:

Blood agar

E. coli was produced 1–4 mm diameter colonies after overnight incubation. The colonies may appear mucoid.

MacConkey agar

E. coli was fermented lactose, produced smooth pink colonies .

- This microbe formed medium sized colonies with a regular margin and convex elevation, lactose fermenter. Catalase positive. Indole from tryptophan +ve (key test). Not only fermented glucose to acid, it also produced gas with bubbles in the Durham tube. This microbe was unable to hydrolyse starch and does not produce amylase. *E.coli* can reduce nitrate to nitrite. This microbe was highly motile. *E.coli* was citrate –ve.

3.9.1.4*Ps.aeruginosa*Identification

Blood agar

*Ps.aeruginosa*was produced large, flat, spreading colonies which are often haemolytic and usually pigment-producing. The pigments diffuse into the medium gave it a dark greenish-blue colour.

MacConkey agar

*Ps.aeruginosa*was produced pale coloured colonies on MacConkey agar. Compared with blood agar, pigment production was less marked.The result of *Ps.aeruginosa*were as follow: none lactose fermenter ,indole negative, none motile, citrate positive.(Cheesbrough, 2006).

3.9.2 Antibiotic Susceptibility Test

All aspects of the Kirby-Bauer procedure were standardized to ensure consistent and accurate results. Because of this, a laboratory must adhere to these standards. The media used in Kirby-Bauer testing was Mueller-Hinton agar at only 4 mm deep, poured into either 100m or 150mm Petri dishes. The pH level of the agar was between 7.2 and 7.4.

3.9.2.1 Incubation Procedure

1.Using an aseptic technique, a sterile swab was placed into the broth culture of a specific organism and then gently the excess liquid was

removed by gently pressing or rotating the swab against the inside of the tube.

2. Using the swab, the Mueller-Hinton agar plate was streaked to form a bacterial lawn. To obtain uniform growth, the plate with the swab in one direction , the platewas rotated 90° and streaked the plate was streaked again in that direction. The rotation was repeated 3 times.

3.The plate was allowed to dry for approximately 5 minutes.

4. An Antibiotic Disc Dispenser was used to dispense disks containing specific antibiotics onto the plate.

5. Using a flame-sterilized forceps, gently each disc was pressed to the agar to ensure that the disc is attached to the agar.

6. Plates were incubated overnight at an incubation temperature of 37°C (Sirdharroa, 2014).

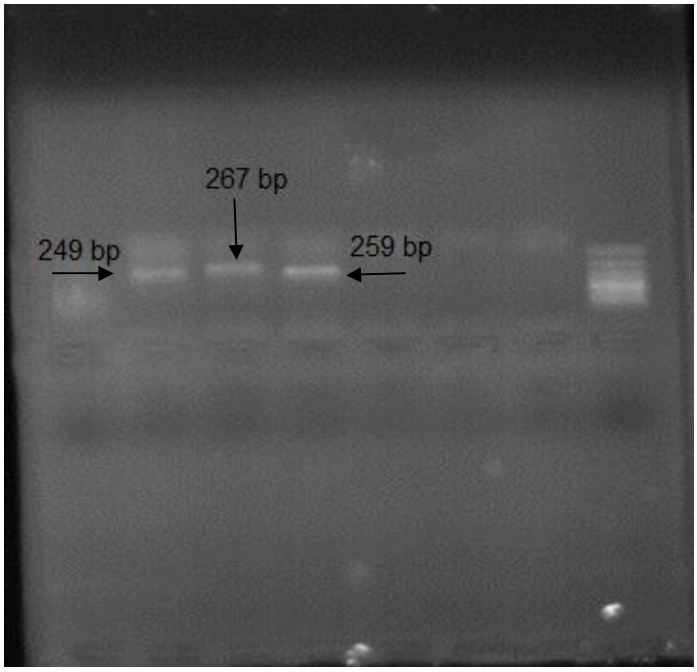
3.10 Statistical Analysis

Statistical software packages (Excel, SPSS 16.0) were used for data management and analysis and data were represented as tables and figures.

4. Results

4.1 PCR Product for Standard Organisms

The 3 standard samples were tested for reconfirmation of the (*S.aureus* ,*E.coli* and *Ps.aurginosa*) and the products of the PCR was run in gel electrophoreses and base pairing(bp) was obtained in(Fig 4-1) .



7 6 5 4 3 2 1

Figure (4-1) PCR product for standard organisms

Lane 1 DNA ladder 100 bp.lane ,Lane 5 *E.coli* 259 bp, lane 6 *S.aureus* 267 bp and lane 7 *Ps.Aurginosa*249bp

4.2 The quality management system(QMS)requirements adoption and compliance

4.2.1 Total QMS requirements compliance

The QMS requirements compliance shows that the adopted requirements for all hospitals was between 34 – 64 %, with average 49% , with range 30% between the upper and lower hospital result (tables 4-1 and 4-2).

Table (4-1) ISO 15189 clauses requirements Comparison between different hospitals laboratories

Study phase		Hospitals laboratories adopted results										
QMS clause	**Required**	**Bahri Renal Centre**	**Bahry**	**Omdurman**	**Albuluk**	**Haj alsafi**	**Bashaier**	**Ahmed gasim**	**Alturkey**	**Ibn sina**	**Alnaw**	**J.ibn auf**
Organization	10	6	6	7	7	4	5	4	7	7	7	6
Personnel	12	8	7	7	5	4	6	4	6	5	8	9
Equipment	18	13	10	11	13	10	8	5	10	8	10	10
Reagents	10	9	6	6	6	6	6	5	8	7	6	8
Pre-analytical	14	10	7	10	10	4	5	6	9	8	9	8
Analytical	12	8	8	8	8	9	8	4	9	8	6	10
Post-analytical	8	8	6	7	6	6	5	3	7	6	6	6
Assuring .Q	14	6	4	8	8	5	1	1	6	2	4	4
Environment	14	13	12	12	12	12	11	9	12	9	12	13
Document	10	3	5	5	5	6	3	2	3	5	4	7
Audit	8	6	5	6	5	6	6	4	6	5	4	6
C. improvement	24	6	6	9	7	8	9	5	6	6	6	12
Total	154	96	82	96	92	80	73	52	89	76	82	99
Percentage %		62	53	62	60	52	47	34	58	49	53	64

Table (4-2) Governmental hospitals laboratories QMS

Hospital laboratory	Required	Adopted	%
Bahri Renal centre	154.00	96.00	62
Bahry	154.00	82.00	53
Omdurman	154.00	96.00	62
Albuluk	154.00	92.00	60
Haj alsafi	154.00	80.00	52
Bashaier	154.00	73.00	47
Ahmed gasim	154.00	52.00	34
Alturkey	154.00	89.00	58
Ibn sina	154.00	76.00	49
Alnaw	154.00	82.00	53
J. ibn auf	154.00	99.00	64

4.2.2 Quality management system clauses requirements application

QMS requirements compliance shows that the average for the percentage of the requirements adoption is 54% (p=0.002) and was significantly different between hospitals. All clauses are significantly different between hospitals except the environment which was significant (p=.02). The Equipmentwas 54.4%. Laboratories personnel have 52.5% from the international standards.. Reagents and Supplies have 66%.The pre-analytical was the main source of false results 55.7%. Assuring quality 32.1% and continuous improvement 30.4%, clauses were the poorer clauses. Documentswere 44%. Internal audit was67.5%(table 4-3).

Table (4-3)Theaverage values adopted for QMS clauses requirement

Clauses	Required	Required total	Adopted total	Average	P-value	%
Organization	10	110	66	6	0.00	60
Personnel	12	132	69	6.3	0.00	52.5
Equipment	18	198	108	9.8	0.001	54.4
Reagents	10	110	73	6.6	0.00	66
Pre-analytical	14	154	86	7.8	0.00	55.7
Analytical	12	132	86	7.8	0.001	65
Post-analytical	8	88	66	6	0.00	75
Assuring .Q	14	154	49	4.5	0.00	32.1
Environment	14	154	127	11.5	0.02	82.1
Document	10	110	48	4.4	0.001	44
Audit	8	88	59	5.4	0.002	67.5
C. improvement	24	264	80	7.3	0.001	30.4
Total	154	1694	917	83.4	0.002	54.2

4.3Results of Laboratories Efficiency

The results of the distributed standard organisms, which was provided for all laboratories was analysed and compared between microscopy identification and susceptibility testing results which was performed.

4.3.1*S.aureus*Results

4.3.1.1 Microscopy

Ten out of eleven laboratories correctly reported Gram-positive cocci and one laboratory did not provide a result for microscopy on this sample.

4.3.1.2 Final Organism Identification:-

Ten out of eleven laboratories correctly reported *S.aureus*. One laboratory failed to provide a result (biochemical test were not available),(table 4-4).

Table (4-4) Final organism identification for *S.aureus*

Bacteria	No	%
S.aureus	10	90.9
No Answer	1	9.1

No answer: - No Gram stain nor biochemical tests were usually carried out. Only culturing and sensitivity test.

4.3.2*E.coli* results

4.3.2.1 Microscopy

Nine laboratories correctly reported Gram-negative bacillus. One laboratory report wrong result of Gram stain. One laboratory failed to provide a response.

4.3.2.2 Final organism identification

Seven laboratories correctly identified *E.coli.* One laboratory incorrectly identified it as*K.pnemoniae*.One laboratory incorrectly identified it as *S.aureus*. One laboratory incorrectly identified it as *Ps.auruginosa*. One laboratory failed to provide a result (table 4-5).

Table (4-5) Final organism identification for *E.coli*

Bacteria	No	%
E.coli	7	63.6
K.pnemoniae	1	9.1
Ps.auruginosa	1	9.1
S.aureus	1	9.1
No Answer	1	9.1

4.3.3 *Ps.aeruginosa* results

4.3.3.1 Microscopy

The ten out of eleven laboratories correctly reported Gram-negative bacillus.

4.3.3.2 Final organism identification

Six laboratoriesout of eleven correctly identified *Ps.aeruginosa*. Two laboratories reported it as*Pseudomonas* (without the spp name). One laboratory incorrectly reported it as *Citrobacter*. One laboratory incorrectly reported it as*E.coli*. One laboratory failed to provide a result.(table 4-6)

Table (4-6) Final organism identification for *Ps.aeruginosa*

Bacteria	No	%
Ps.aeruginosa	6	54.6
E.coli	1	9.1
Pseudomonas	2	18.2
Citrobacter	1	9.1
No Answer	1	9.1

As summationfor the three standard organisms, 3 out of 33 were false Gram stain results9.1%, 9 out of 33 final identification results were false with percentage 27.3%, the false results were distributed between biochemical results and nomenclature(table 4-7).

Table (4-7) False and true identification results for the three standard organisms:-

	Gram stain		Identification results	
	No	**%**	**No**	**%**
True results	30	90.9	24	72.7
False results	3	9.1	9	27.3
Total	33	100	33	100

4.4 Antibiotic Susceptibility Tests:-

All the laboratories use disc-diffusion method (Kirby-Bauer method). They used Muller Hinton agar media. One hospital has multi-disc for Gram positive and gram negative. Two laboratories report the result with the zone diameter while the rest adopt (R=Resistant, S=Sensitive, I=Intermediate). According to standard antibiotics susceptibilitychart .

4.4.1 Antibiotic Susceptibility Tests for *S.aureus*

The total number of antibiotics used were nineteen. Ciprofloxacin, amikacin and gentamicin were used by nine laboratories. One laboratory used four antibiotics, and another laboratory used five antibiotics, one laboratory used seven antibiotics, eight laboratories used eight antibiotics for susceptibility testing. Ceftazidime has three (false) sensitive and five resistant (true) results. Cefexime has four (true) sensitive and two (false) resistant results. Amikacin has eight (true) sensitive and one (false) intermediate result. Cefuroxime has four (true) sensitive and one (false) intermediate result. Augmentin has three (true) sensitive, one (false) resistant and one (false) intermediate result.(table4 -8)

Table (4-8) Antibiotic susceptibility test for *S.aureus*

Antibiotics	S	R	I	Nottested	Total
Vancomycin	1	0	0	10	11
Tetrcycline	1	0	0	10	11
Sulfamethazole	1	0	0	10	11
Norfloxacin	2	0	0	9	11
Nitrofrontion	1	0	0	10	11
Linofcoxacin	1	0	0	10	11
Ienomycin	1	0	0	10	11
Gentamicin	9	0	0	2	11
Cotrimexazole	1	0	0	10	11
Ciprofloxacin	9	0	0	2	11
Cetazidime	3	5	0	3	11
Cefuroxime	4	0	1	6	11
Ceftazin	0	1	0	10	11
Ceftriaxone	6	0	2	3	11
Cefotaxime	1	0	0	10	11
Cefixime	4	2	0	5	11
Augmentin	3	1	1	6	11
Ampecellime-cellpadim	1	0	0	10	11
Amikacin	8	0	1	2	11

4.4.2 Antibiotic susceptibility tests for *E.coli*

The total number of antibiotic used was fourteen. Amikacin was used by ten laboratories. Ciprofloxacin was used by nine laboratories. Gentamicin and ceftazidime was used by eight laboratories. Ceftriaxone was used by seven laboratories. Cefuroxime and cefexime was used by six laboratories. Sulfamethazole, norfloxacin, cefotaxime, ampicillin, penicillin, astroneme, and chlorenphenicol were used by one laboratory.

Ceftazidime is the only one antibiotic that has false sensitivity result by two laboratories; six laboratories issue the result as sensitive while two adopted the result as intermediate (table 4-9).

Table (4-9) antibiotic susceptibility test for *E.coli*

Antibiotics	S	R	I	Nottested	Total
Ciprofloxacin	9	0	0	2	11
Amikacin	10	0	0	1	11
Gentamicin	8	0	0	3	11
Cefriaxone	7	0	0	4	11
Cetazidime	6	0	2	3	11
Cefuroxime	6	0	0	5	11
Cefixime	6	0	0	5	11
Sulfamethazole	0	1	0	10	11
Norfloxacin	1	0	0	10	11
Cefotaxime	1	0	0	10	11
Ampicillin	1	0	0	10	11
Penicillin	1	0	0	10	11

4.4.3Antibiotic Susceptibility Tests for *Ps.aeruginosa*

The total number of antibiotic used was fourteen. Amikacin was used by ten laboratories. Ciprofloxacin, ceftazidime and ceftriaxone was used by eight laboratories. Gentamicin was used by seven laboratories. Cefexime used by six laboratories. Cefuroxime was used by five laboratories. Augmentin was used by four laboratories. Chlorenphenicol was used by three laboratories. Amidium, penicillin, astroneme, norfloxacin and nitrofrontoin was used by one laboratory. Ceftriaxone has seven (true) resistant results and one (false) sensitive result. Chlorenphenicol has two (true) resistant results and one (false) sensitive result. Ceftazidime has five (true) resistant results and three (false) sensitive results. Cefexime has five (true) resistant results and one (false) sensitive result. Augmentin has three (true) resistant results and one (false) sensitive result (table4-10)

Table (4-10) antibiotic susceptibility test for *Ps.aeruginosa*

Antibiotics	S	R	I	Not tested	Total
Ciprofloxacin	8	0	0	3	11
Amikacin	10	0	0	1	11
Gentamici	7	0	0	4	11
Ceftriaxone	1	7	0	3	11
Amidium	1	0	0	10	11
Penicillin	1	0	0	10	11
Astroneme	1	0	0	10	11
Chlorenphenicol	1	2	0	8	11
Cetazidime	3	5	0	3	11
Cefuroxime	5	0	0	6	11
Cefixime	1	5	0	5	11
Augmentin	1	3	0	7	11
Norfloxacin	1	0	0	10	11
Nitrofrontion	1	0	0	10	11

As generalfor antibiotic susceptibility tests there were (13-27.6%) false error. No laboratory has stable and documented programme for internal quality of sensitivity testing(table 4-11).

Table (4-11) Errors of antibiotics susceptibility tests:-

	True	Error	Total
No	34	13	47
%	72.4	27.6	100

5. Discussion

5.1 Discussion

The main purpose of this study was to assess the Microbiology Laboratory Quality Control in the governmental microbiology laboratories in Khartoum State. This purpose was done by measuring the efficiency, applying the standards of ISO 15189:2007 checklist clauses, and checking the sensitivity test results of the standard organisms (ATCC/NCTC type).

For identification to three standard organisms were used differential staining (Gram staining). Morphological identification (colonial morphology, cell shape and cell size), and biochemical tests, for sensitivity test thedisk diffusion antibiotic sensitivity testing was used.

Study was conducted during the period from January to March 2016.

The application of ISO 15189:2007 clauses showed that the adopted percentage was between 34% to 64%, the situation analysis of the laboratories under study reveals that these laboratories were poor on all clauses of international standards organization 15189 requirements, this finding was agreed with a study which was done by Gurol*et al*.(2011) they found that it was very difficult to apply the standards of ISO 15189:2007 and accreditation is a very hard process and should be tackled with a teamwork.

The identification results were received for the three standard organisms (NCTC/ ATCC) types; they were having major errors in identifications of these organisms:

For *S.aureus*, one laboratory failed to provide a result, this laboratory had no biochemical tests which lead the microbiologist to identify the organism.For*E.coli,* one laboratory reported the microscopy result as Gram positive Cocci while the organism was Gram negative bacilli, this result considered as major error. In the final identification results: 4/11

(36%) false results were reported as follows: one as *K.pnemoniae,* another one report*Ps.aeruginosa,* another laboratory report *Staph*, and the last one failed to provide a result as mentioned before with *S.aureus* this laboratory did not have the biochemical tests to identify the organism.

For*Ps.aeruginosa,* the final identification results had 5/11 (45.5%) false results and they were reported as follows: one laboratory failed to provide a result as mentioned before with the previous organism (have no biochemical set), one laboratory report the result as *Citrobacter*, one laboratory reported *E.coli*, two laboratories had a nomenclature error and reported the genus without the species name *(Pseudomonas*).This agreed with a study that donin Alberta by Church *et al*.(2000) they found that the microbiology laboratory restructuring will have adverse effects on the quality of complex testing if experienced technologists are not retained and services are not medically supervised..

The antibiotics susceptibility tests were done and the results received, were observed for some errors. Firstly there was a laboratory use of multidisc for Gram positive and multidisc for Gram negative, which considers as wrong practice. As general these laboratories had no unique policy for choosing the antibiotics, this finding agrees with another study done by Baron *et al*.(1996)they found that there was no unique policy for choosing the antibiotics. As for *S.aureus*: Ceftazidime has three false sensitive results, Cefexime has two falseresistant results, Cefuroxime has one false intermediate result, Augmentin one false resistant and one false intermediate result, this indicate that the performance of susceptibility tests have not yet reached the level seen for identification.

As for *E.coli* there were two errors with ceftazidime which was issued as intermediate by two laboratories while it is actually sensitive.

As for *Ps.aeruginosa*, Ceftriaxone had one false sensitive, ceftazidime had three false sensitive results, Cefexime had one false sensitive result, and Augmentin had one false sensitive result.

This finding was agreed with the finding obtained by Bell*et al*.(2009),and Kumasaka*et al*.(2001), they found that the performance as generally for identification and susceptibility test was worse.

5.2 Conclusion

This study concludes that:

. There are no uniform policies for identification and susceptibility tests in the state.

. Microbiology laboratory in Khartoum state were not well equipped and work as bacteriology lab, no immunology, no molecular biology and no even mycology inside these laboratories.

. Instruments are not checked daily (no charts) and there no preventive maintenance programme.

. The pre-analytical process is the main source of false results.

5.3 Recommendations

.Surveys and researches should carry out periodically to assess the performance of the microbiology laboratories in Khartoum state.

. All laboratories need to review their quality management system, policies, procedures, and processes to control the testing activities

. Personnel should in a continuous education programme and there should be a clear job description.

.Laboratory personnel need to be well trained and need continuous follow up to encourage them to apply the standards.

. Uniform procedures and guidelines should be built and distributed to the laboratories

. A set of reference strains for monitoring the performance of media and reagents should be available and used routinely (IQC).

. Laboratories should be well equipped and there must be stable programme for maintenance.

. Accreditation is important and applicable, so there should be strategiesto how can beapplied on these laboratories, and what to do to meet the international standards.

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Appendices

Appendix I:

STD organisms sourced from NCTC

Appendix II

STD organisms sub-cultured in nutrient agar

Appendix III

ISO 15189 checklist

Laboratory name: …… organization: ……..……

Auditor(s)…….Date of visit ……

1.Laboratory Organization							
NO	**QMS Standard**	**Y**	**P**	**N**	**Adopted**	**required**	**%**
1	**Laboratory shall have the organizational and management structure**					**2**	
2	**Appointment of quality manager with delegated responsibility.**					**2**	
3	**Appointment deputies for key function**					**2**	
4	**Laboratory shall be equipped with needed resources**					**2**	
5	**Laboratory shall have document review meeting**					**2**	
						10	
2. laboratory personnel							
1	**Laboratory shall have clear policy for personnel management**					**2**	
2	**Laboratory shall have adequate staff resources**					**2**	
3	**Laboratory management shall have personnel job description**					**2**	
4	**Laboratory shall be a continuing education program available to all staff**					**2**	
5	**Laboratory shall have procedure to control personnel performance**					**2**	
6	**Laboratory shall maintain records of all personnel**					**2**	
						12	

3. LABORATORY EQUIPMENT							
		Y	P	N	Adopted	required	%
1	Laboratory shall be furnished with all equipment required for services					2	
2	Equipment shall be calibrated and maintained (document).					2	
3	Instruments shall have documented maintenance.					2	
4	Equipment shall be maintained in a safe working condition.					2	
5	Equipment shall be operated by authorized personnel only					2	
6	Equipment shall have maintenance and user's manual.					2	
7	Equipment's shall have labels to indicate verifications.					2	
8	Defected equipment's shall be taken out of work station.					2	
9	Equipment shall be safeguarded from adjustments or tampering.					2	
						18	
4. laboratory reagents and supplies							
1	Laboratory shall define and document its policies and procedure for selection and use of equipment and consumable supplies					2	
2	Laboratory shall have specifications for supplies, and reagent					2	
3	Purchased equipment and consumable supplies shall not be used until they have been verified					2	
4	The purchased reagent and supplies should be stored in optimum condition					2	
5	Laboratory shall have developed procedure to control supplies consumptions.					2	
						12	

5.1 pre-analytical process							
1	**Laboratory shall have instructions for the proper specimen collection**				**2**		
2	**Laboratory shall have procedure for sample preparation**				**2**		
3	**Sample potions shall also be traceable to the original primary sample**				**2**		
4	**Laboratory shall monitor the transportation of samples**				**2**		
5	**Criteria shall be developed for acceptance or rejection of primary sample.**				**2**		
6	**Laboratory shall have documented for rejection of inappropriate sample**				**2**		
7	**Laboratory shall have a procedure for storage primary sample.**				**2**		
					14		
5.2 Analytical							
1	**Laboratory shall be used procedures are those that have been published in established/authoritative textbook. Or internationally accepted.**				**2**		
2	**Laboratory shall have SOPs**				**2**		
3	**Laboratory shall be reviewed of procedures at least once in twelve months and documented**				**2**		
4	**If the laboratory intends to change an examination procedures laboratory staff should be informed**				**2**		
5	**All procedure should be documented and be available on workstation.**				**2**		
6	**Laboratory management in consultation with the requesters shall establish turnaround times for each examination**				**2**		
					12		

5.3 post analytical							
NO	**QMS Standard**	**Y**	**P**	**N**	**Adopted**	**required**	**%**
1	**Authorizedpersonnel systematically review the results and signature.**					**2**	
2	**Laboratory shall have issuing the result in tow copies.**					**2**	
3	**Result should be delivered to the right patient.**					**2**	
4	**Critical result should be delivered to the doctor immediately**					**2**	
						8	
6. assuring quality of examination procedure							
1	**Laboratory shall have quality manual.**					**2**	
2	**Laboratory shall design internal quality control systems.**					**2**	
3	**Laboratory shall have corrective action records where internal quality control out of range.**					**2**	
4	**Laboratory shall participate in EQA.**					**2**	
5	**Laboratory management shall monitor the result of external assessment and participate in implementation of corrective actions**					**2**	
6	**Documentation of reagents, procedures or the examination system validation.**					**2**	
7	**For those examinations performed using different equipment; there shall be a defined mechanism for verifying the comparability of results throughout the clinically appropriate intervals.**					**2**	
						14	

7. Environmental condition							
1	**Laboratory shall have space quite enough.**					**2**	**%**
2	**laboratory design suitable environment.**					**2**	
3	**Laboratory shall have separation between incompatible departments**					**2**	
4	**Laboratory shall be controlled temperature of refrigerator.**					**2**	
5	**Sample shall be storage at suitable condition.**					**2**	
6	**Work area shall be clean and well maintained.**					**2**	
7	**Laboratory shall have procedure for storage and destroy hazard samples and also have procedure for prevent an environment.**					**2**	
						14	
8. documents control							
1	**All documents relevant to the quality management system shall be uniquely identified**					**2**	
2	**Quality documents shall be included title, edition, or current revision date or revision number of pages, authority for issue and source identification.**					**2**	
3	**Laboratory shall have procedure for safe the valid records.**					**2**	
4	**All records shall be legible and stored such that they are readily retrievable.**					**2**	
5	**Laboratory shall have policy that defines the length of time various records pertaining to the quality management system.**					**2**	
						10	

9. INTERNAL AUDIT							
1	**The laboratory shall be conducted an internal quality audit.**					**2**	**%**
2	**The laboratory shall have recorded results for internal audits.**					**2**	
3	**The laboratory shall adopted appropriate corrective and preventive action.**					**2**	
4	**The result of internal audit shall be reviewed by laboratory management.**					**2**	
						8	
10. continual improvement							
1	**Laboratory shall be reviewed quality management system periodically**						
2	**Management review shall take account of follow-up previous management reviews, status of corrective and required preventive action and outcome of external quality assessment.**						
3	**The laboratory shall be continually reviewed the process of work of the aspect of completeness and accuracy.**						
4	**The laboratory shall audit result work according to purpose and objective of organization.**						
5	**In case of mistake which is affect to policies, procedure or quality management system, associated activities shall be evaluated.**						
6	**The laboratory shall submitted reports to management board.**						
7	**The laboratory shall have development quality system activities between organization and the lab.**						
8	**Procedure for preventive action shall include the initiation of such actions and application of controls to ensure that they are effective.**				**2**		

9	**Laboratory shall have a policy and procedure to detect and control non conformance activities.**				**2**		
10	**Laboratory shall implement quality indicators.**				**2**		
11	**The laboratory shall have a policy and procedures for the resolution of complain or feed back reviewed from clinicians, patients or other parties.**				**2**		
12	**The laboratory shall check the customer satisfaction.**				**2**		
					24		

Checklist assessment keys:

Y= means the standard was fully conform to the requirements. (Two marks)

P= means the standard was partially conform to the standard. (One mark)

N= means the standard was not conform to standards. (0 mark)