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Review

Value of matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry in clinical microbiology and infectious diseases in Africa and tropical areas

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Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MALDI-TOF MS) is a revolutionary technique with multiple applications. Its use in clinical microbiology is now becoming widespread as the method is an easy, rapid, effective, accurate, and cheap way to identify cultured bacteria and fungi. It is, therefore, an ideal tool to replace conventional methods still used in Africa and tropical areas for routine microbiological diagnosis. The recent installation of a MALDI-TOF MS for diagnostic purposes in a hospital in Senegal has confirmed that this tool is not only valuable but also robust in tropical Africa, providing further evidence that this technique should be widely distributed there. However, despite its value for clinical microbiology in Africa, the acquisition and installation of MALDI-TOF MS is subject to several constraints. This review provides general information on aspects of MALDI-TOF MS. The specific aspects and constraints observed in Africa and tropical countries are also addressed with suggestions for appropriate solutions.

Key words: Microorganism, infectious diseases, quick identification, matrix-assisted laser desorption-ionization time-of-flight, matrix assisted laser desorption ionization-time of flight (MALDI-TOF).

INTRODUCTION

Cardiovascular diseases are the leading cause of death in developed countries, while in Africa and low-income countries, thousands of deaths linked to infectious diseases are recorded every year (Prost, 2000; Lopez et al., 2000; Bryce et al., 2005; Williams et al., 2002).

Against this backdrop of the high incidence of infectious diseases, including emerging and reemerging pathogens (Desenclos and De Valk, 2005), improving tools for the

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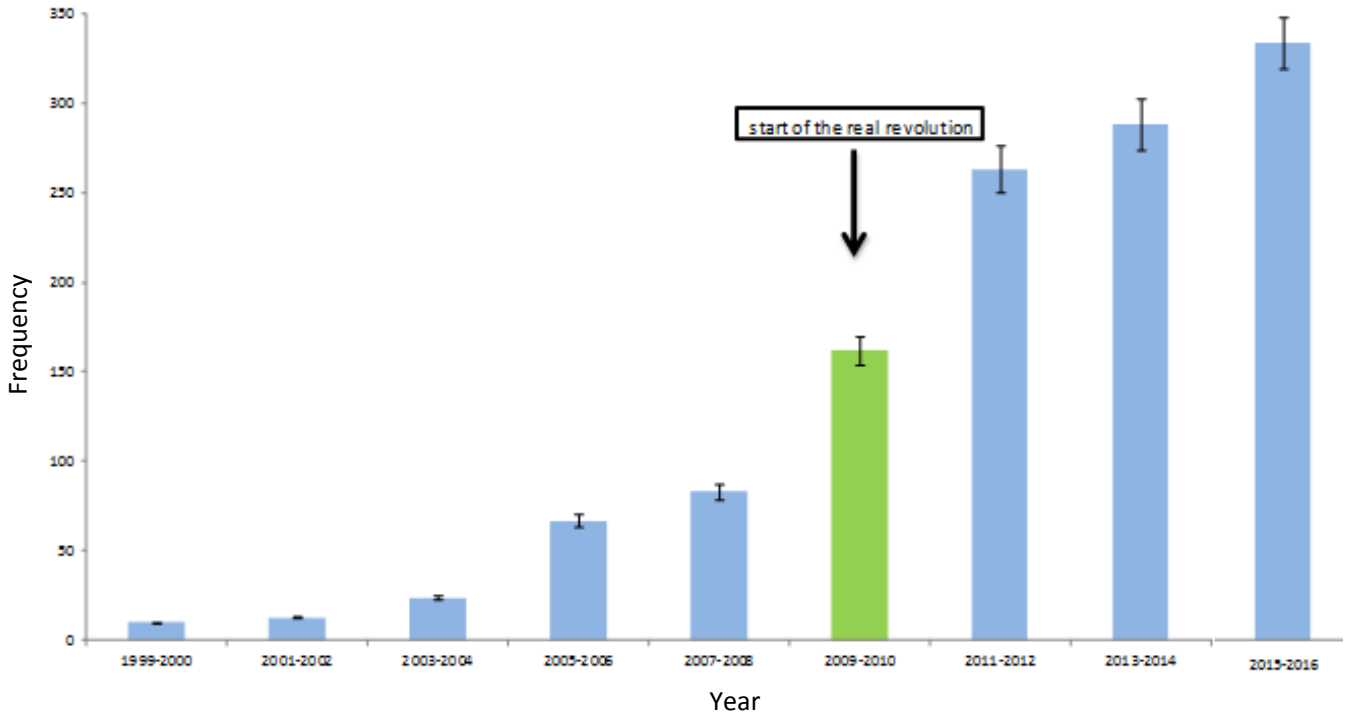

Figure 1. Increasing number of publications related to MALDI-TOF MS applications in medical microbiology from 1999 to 2014. It shows also that 2009 marks the massive use of MALDI-TOF MS in clinical microbiology laboratories. The following Mesh terms through bibliographic NCBI database were used to built this graph: ("spectrometry, mass, matrix-assisted laser desorption-ionization"(MeSH Terms) OR ("spectrometry""(All Fields) AND "mass"(All Fields) AND "matrix-assisted"(All Fields) AND "laser" (All Fields) AND "desorption-ionization"(All Fields]) OR "matrix-assisted laser desorption-ionization mass spectrometry" (All Fields) OR "maldi" (All Fields) AND "tof" (All Fields) AND ("microbiology" (Subheading) OR "microbiology" (All Fields) OR "microbiology" (MeSH Terms) AND ("1999/01/01" (PDAT): "2014/12/31" (PDAT).

identification of microorganisms in clinical microbiology laboratories is urgently required.

In African countries, routine diagnostic methods are generally based on culture media, followed by growth characteristics and biochemical patterns. These steps are fastidious, requiring large quantities of expensive reagents and *a priori* knowledge of the isolated microorganism; identification may take place after several hours or days, depending on the microorganism, and even then is sometimes inaccurate (Seng et al., 2009).

Recently, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has enabled a revolution in the routine work of clinical microbiology laboratories with the quick, inexpensive, and accurate identification of bacteria and fungi. Without any *a priori* knowledge, it is possible to quickly adapt first line anti-infective treatment as the best possible treatment (Courcol, 2009). MALDI-TOF MS is having a real impact on global health and its implementation will be of great value in Africa and other tropical areas, as recently shown in Senegal, where its broad applicability and robustness have been recently proven (Fall et al., 2015; Lo et al., 2015).

Here, we will review the general aspects of MALDI-TOF MS but will also focus on the specific aspects and constraints observed in Africa and tropical countries. We will also propose appropriate solutions.

GENERAL ASPECTS OF MALDI-TOF MS TECHNOLOGY

In 1975, the scientific literature began to combine MS with pyrolysis for the detection of bacterial proteins (Intelicato-Young and Fox, 2013). In 2009, a new revolution in clinical microbiology began when the efficiency of MALDI-TOF MS for the routine identification of bacteria was demonstrated with a correct identification of 95 and 84% of the genus and species levels, respectively, for 1,660 bacteria (Seng et al., 2009; Seng et al., 2010).

Since then, an explosion in scientific publications on the use of MALDI-TOF MS in clinical microbiology has been observed (Figure 1), supporting the fact that the method is a fast and reliable means of identifying microorganisms and is clearly more efficient than conventional methods (Eigner et al., 2009; Blondiaux et al., 2010). It has been estimated that ten bacterial strains can be identified in parallel in less than 15 min with MS, while it takes more than 360 min to do so using

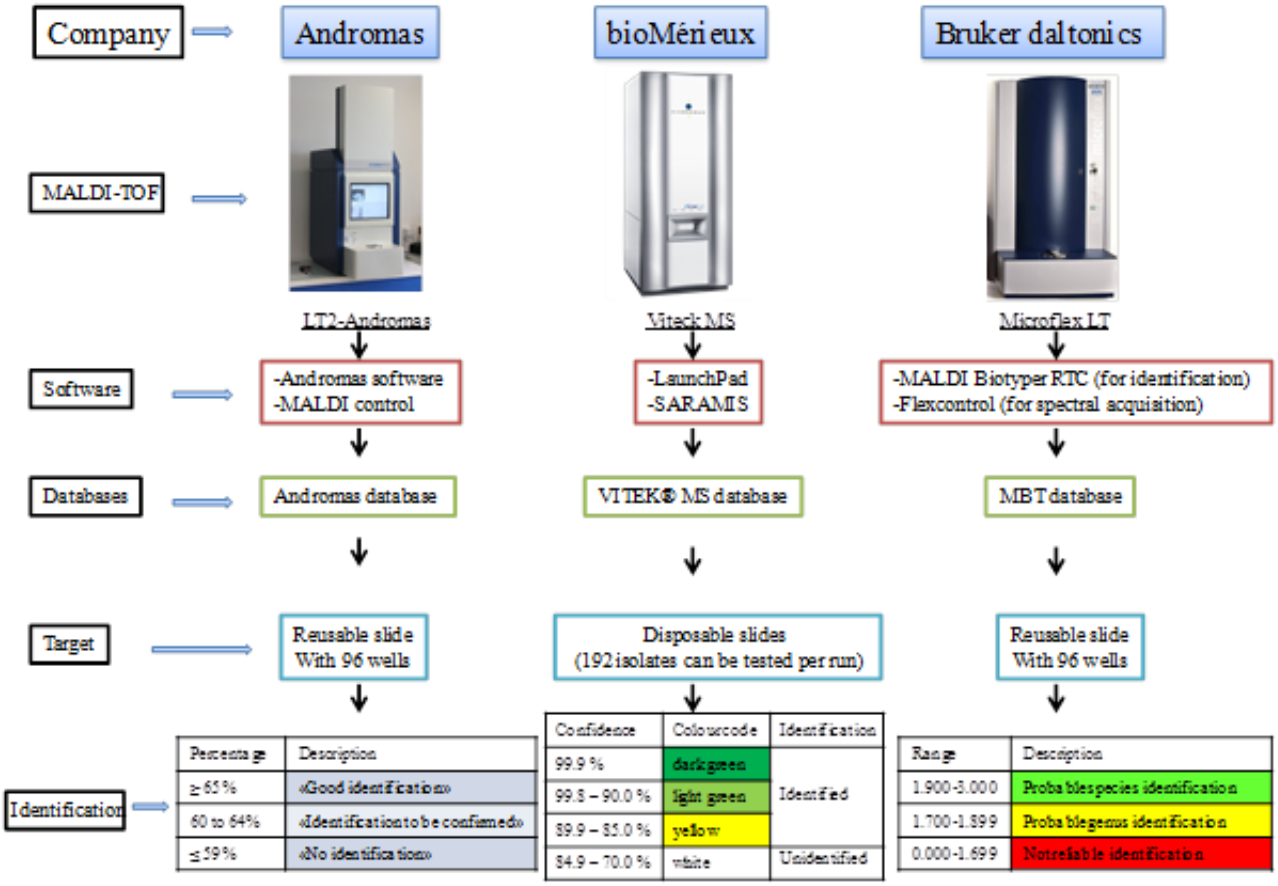

Figure 2. The various MALDI-TOF MS instruments are currently commercialized for the identification of microorganisms in clinical laboratories. The LT2-Andromas, Vitek MS, and MALDI Biotyper have been accredited for identification purposes in clinical microbiology laboratories under EU directive EC/98/79 in several European countries. The VITEK® MS and the MALDI Biotyper were cleared by the US Food and Drug Administration (FDA) for the identification of cultured bacteria and, in the case of the former system, yeast in 2013.

conventional automated systems (Biswas and Rolain, 2013; Cherkaoui et al., 2010).

Currently, three MALDI-TOF mass spectrometers are on the market (Figure 2); the Andromas system (Paris, France) (Bille et al., 2012), the Microflex LT (Bruker Daltonics, Heidelberg, Germany, in collaboration with Becton Dickinson, Franklin Lakes, NJ, USA) (Lee et al., 2015; Saffert et al., 2011), and the VITEK® MS (bioMérieux, Marcy l'Etoile, France) (Patel, 2013).

For bacterial and fungal identification, one isolated colony is picked and directly deposited on a well of a MALDI-TOF plate, preferentially in duplicate, as the deposit is crucial for accurate identification (Figure 3) (Fenselau and Demirev, 2001). This preparation must then be overlaid with a matrix solution (solution with alpha-cyano-4-hydroxycinnamic acid, acetonitrile, trifluoroacetic acid, etc.), and air dried at room temperature (about five minutes) to permit co-crystallization (Shunsuke et al., 2014) before placing the plate in the MALDI-TOF instrument for analysis. Identification is achieved by comparing the spectra of analyzed species against the reference spectra present in the MALDI-TOF database (Coltella et al., 2013; Martiny et al., 2012).

Identification robustness depends on the richness of the databases, which have been regularly and substantially updated since 2009 (Seng et al., 2010). The database provided with the Vitek MS installed in Dakar, is annually updated by bioMérieux. Indeed, commercial database are regularly updated and released (approximately one time per year). Besides, depending on the MALDI-TOF mass spectrometer, database can be incremented directly with spectra from local bacteria paving way for data with local epidemiology. Specific database can also be created for entomology (Sambou et al., 2015).

Recently, Tran et al. (2015) performed a huge study to evaluate the cost savings of implementing routine microbiological identification by MALDI-TOF MS (bioMérieux Vitek, Durham, NC, USA) in their laboratory.

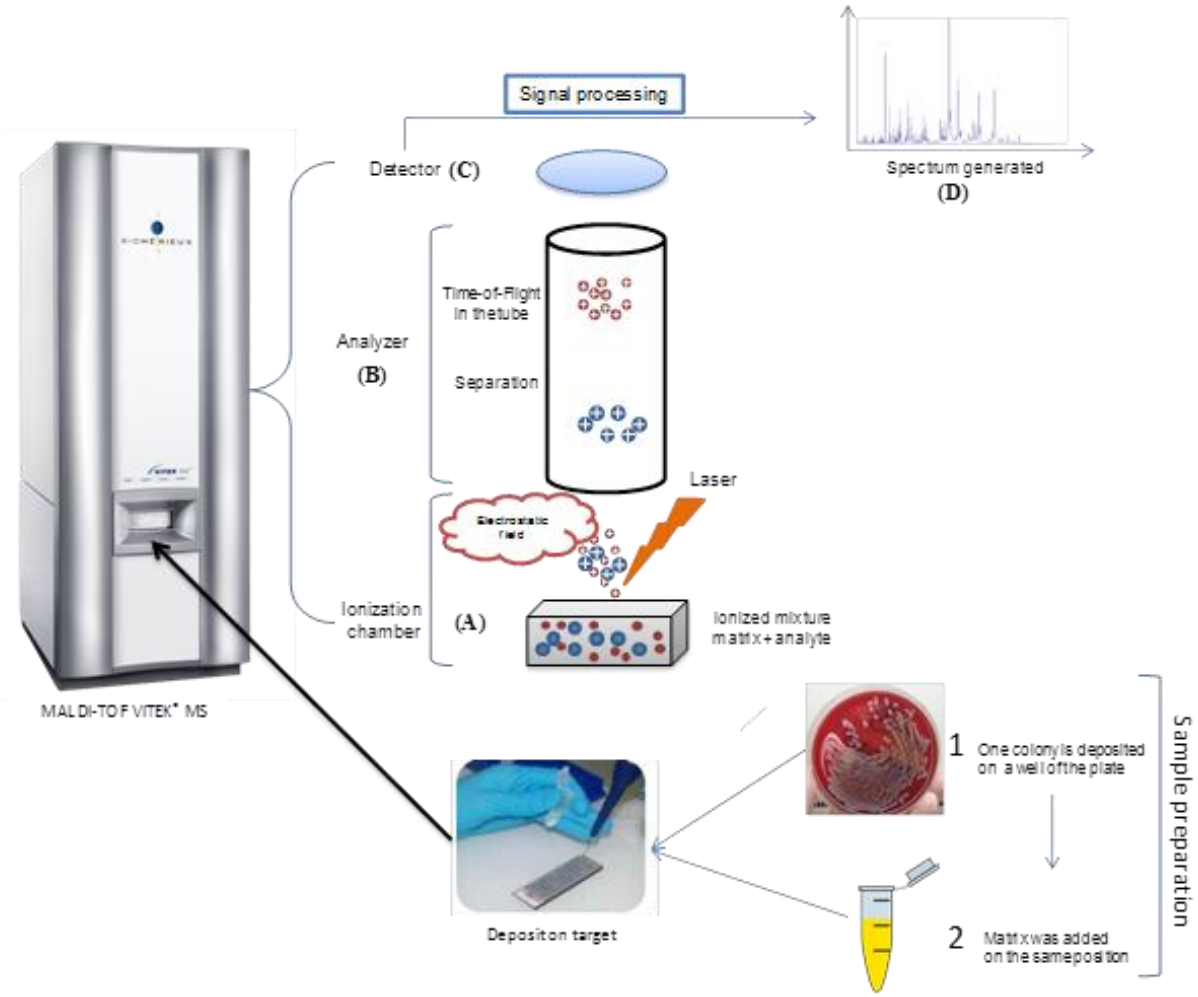

Figure 3. MALDI-TOF MS's operating principle and the sample preparation step for identification. The principle of this measurement is based on the ability of an electric and/or magnetic field to deflect a flow of ions, each with a mass and a charge proportional to their trajectories. Overall, mass spectrometry can be divided into three steps: the ionization chamber that produces ions in the gas phase (A), the analyzer which selects ions by mass-to-charge ratio (m/z) (B), and the detector that converts the ionic current into electric current (C). Bombing with a laser beam generates ions in the ionization chamber. These ions are accelerated into an electric field which directs them to the analyzer that separates them according to their time-of-free flight (TOF: Time-Of-Flight). The smaller molecules grasp the detector first, followed by the biggest, according to the m/z ratio. Those which have the same m/z ratio are then separated by an electrostatic mirror. The detector converts the received ions into electrical current which is amplified and digitized (D).

Overall, reagent costs for the conventional methods averaged $3.59 per isolate, while those for MS were $0.43. The use of MS was equated to a net saving of $69,108.61 (87.8%) in reagent costs annually. When technologists' time and maintenance costs were included, conventional identification cost would be $142,532.69 versus $68,886.51 with MS, resulting in a laboratory saving of $73,646.18 (51.7%) annually. They also estimated that the initial cost of the instrument at their usage level would be offset in about three years (Tran et al., 2015). Comparing MALDI-TOF MS to other identification methods which is usually used in microbiology laboratories shows that it is very cost-effective in terms of reagent cost and working time (Table 1) (Musser, 2014).

The direct identification of microorganisms in specimens such as blood cultures, urine, or cerebrospinal fluid has been proposed using home-made (Segawa et al., 2014; Ferreira et al., 2010; Yonetani et al., 2016; Foster, 2013; Ferroni et al., 2010; Ferreira et al., 2011) or commercial kits for blood cultures (Jamal et al., 2013; Haigh et al., 2013; Nonnemann et al., 2013).

Currently, direct identification is mainly available for blood cultures after a pre-incubation step but it allows the quick identification of the involved microorganism and the opportunity of considerably earlier treatment adaptation,

Table 1. Cost comparisons between MALDI-TOF MS and tests used in clinical microbiological laboratories for bacterial identification.

Tests	Reagents cost only by test	Estimated staff time needed to perform test (h)	Estimated cost in staff time per test	Total cost per test (staff and reagents)
16S rDNA sequence analysis	$11.97	03	$218.87	$230.84
Real-Time PCR	$4.99	0.5	$36.47	$41.47
Individual biochemical test	$0.99	0.15	$10.94	$11.94[1]
MALDI-TOF MS	$0.50	0.5	$36.47	$36.98

[1]This represents only one biochemical test not a panel of tests required for bacterial identification.

with a direct clinical impact (Kohlmann et al., 2015).

In addition, MALDI-TOF MS is a promising, easy, inexpensive, and rapid tool for investigating an outbreak (Croxatto et al., 2012; Gaia et al., 2011; Fujinami et al., 2011; Williamson et al., 2008). For example, the epidemiological investigation of a nosocomial outbreak of multidrug resistant *Corynebacterium striatum* showed that all outbreak-related strains are clustered in a single clone with a MALDI-TOF MS dendrogram (Verroken et al., 2013). It has also enabled the accurate and reproducible discrimination of major methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complexes observed in outbreaks, belonging to strains prepared with the same extraction protocol (Wolters et al., 2011; Josten et al., 2013).

MALDI-TOF MS has also made it possible to differentiate the five most frequently-isolated *Salmonella enterica* serovars (Enteritidis, Typhimurium, Virchow, Infantis, and Hadar) (Dieckmann and Malorny, 2011) as well as to identify *Escherichia coli* pathotypes (Clark et al., 2013; Barbuddhe et al., 2008). The validity of MALDI-TOF MS for typing extended-spectrum β-lactamase-producing *E. coli* in a previously published nosocomial outbreak was recently assessed (Egli et al., 2015). Thus, all these data clearly show that MALDI-TOF MS has a promising future in the epidemiological surveillance of infectious diseases (Doern and Butler-Wu, 2016).

Africa is prey to endemic diseases such as malaria. This is why the use of rapid and effective control methods could permit the prevention and control of vector-borne diseases. Several studies have shown that MALDI-TOF MS has also enabled the rapid detection of arthropod vectors, such as ticks, mosquitoes, fleas (Yssouf et al., 2014), phlebotomine sand flies (Mathis et al., 2015), and *Culicoides* without any expertise or skills in entomology (Sambou et al., 2015; Yssouf et al., 2013a).

Recently, the utility of MALDI-TOF MS for a dual identification of tick species and bacteria has been demonstrated. Intracellular *Rickettsia* spp. has been detected using MALDI-TOF MS in ticks (Yssouf et al., 2015), as well as *Borrelia crocidurae* in *Ornithodoros sonrai* ticks (Fotso et al., 2014). This concept offers new perspectives for monitoring other vector borne diseases that present public health concerns.

Finally, MALDI-TOF MS has also facilitated the identification of meat origin in raw and processed meats, and fish in culinary preparations (Mazzeo et al., 2008; Flaudrops et al., 2015). Key stages in the use of MALDI-TOF MS for identification purposes other than microbial purposes are summarized in Table 2 (Yssouf et al., 2014; Yssouf et al., 2013a; Mazzeo et al., 2008; Kaufmann et al., 2012; Yssouf et al., 2013b; Steinmann et al., 2013; Flaudrops et al., 2015).

VALUE OF MALDI-TOF MS IN AFRICA

Bacterial and fungal identification in Africa

Conventional biochemical identification methods (Figure 4) are in standard use in Africa, although performance limitations sometimes exist (Patel, 2013; Samb-Ba et al., 2014). Storage of the various reagents requires strict conditions and compliance with expiration dates. Difficulties with cold storage are also observed, which can have a real impact on reagents. Reagent supply issues have also been experienced. Finally, identification is often based on interpretation of the few biochemical tests available, sometimes leading to inaccurate identification which can have a clinical impact on patient treatment. Thus, use of new-generation technologies such as MALDI-TOF MS may resolve many of these difficulties. The low cost, speed, and accuracy of identification without prior knowledge supports the claim that the use of

Table 2. Key stages in the use of MALDI-TOF MS for identification purposes other than microbial.

References	First use of MALDI-TOF MS for identification purpose	Year
Mazzaeo et al.	Fish	2008
Kaufmann et al.	Culicoides	2012
Yssouf et al.	Ticks	2013[a]
Steinmann et al.	Ceratopogonid and culicid larvae	2013
Yssouf et al.	Mosquitoes	2013[b]
Yssouf et al.	Fleas	2014
Flaudrops et al.	Meat from raw and processed meat in culinary preparations	2015

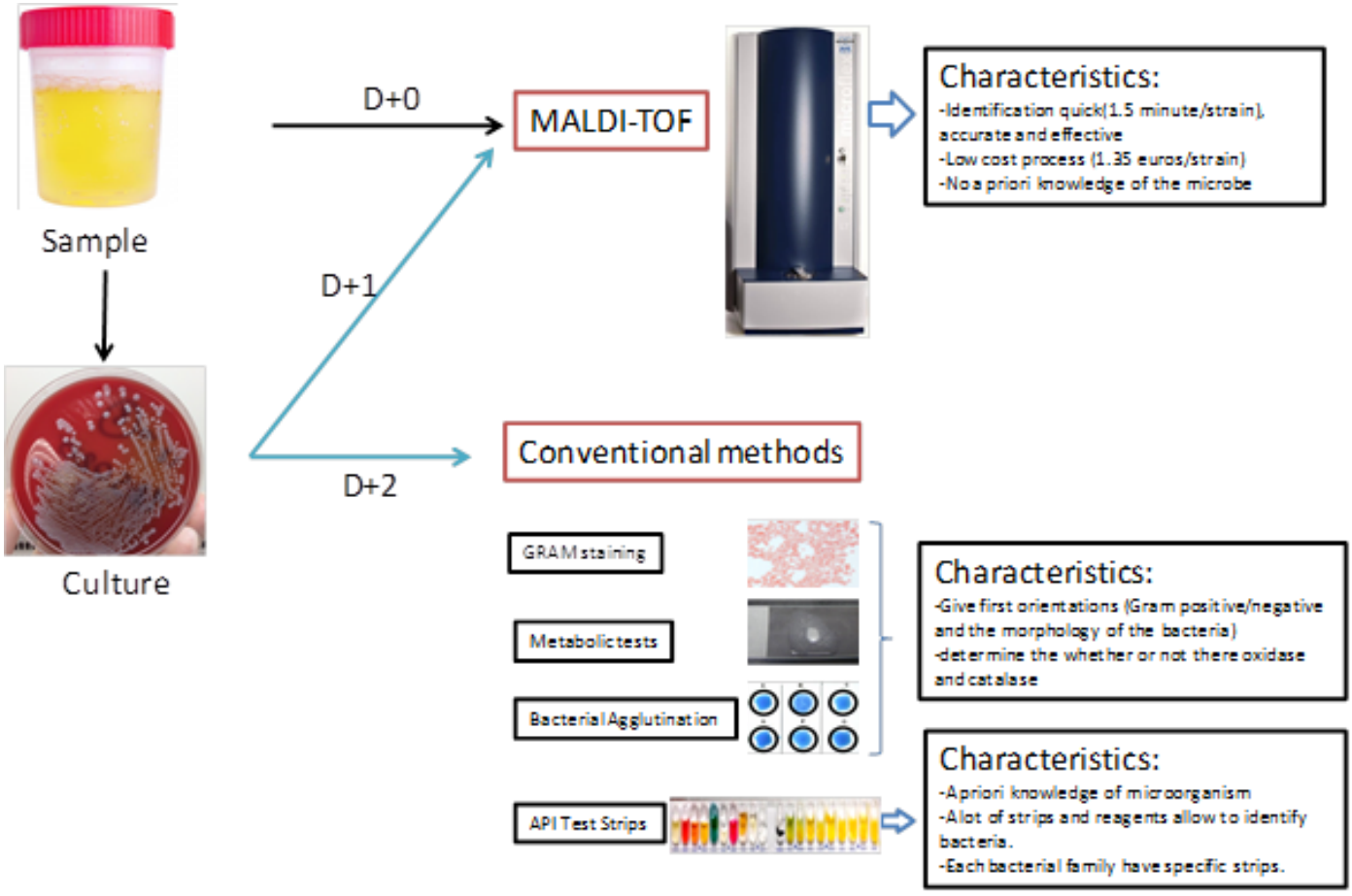

Figure 4. MALDI-TOF MS performance compared to conventional methods is routinely used in some clinical laboratories in Africa.

MALDI-TOF MS will help in microbiology laboratories in Africa (Cherkaoui et al., 2010; Bizzini and Greub, 2010). When we implemented a VITEK® mass spectrometer RUO (bioMérieux, Marcy l'Etoile, France) in Senegal (Hôpital Principal de Dakar) in 2012, conventional methods such as API strips were immediately stopped. In just ten months, the instrument correctly identified 2,082 bacteria and fungi at the species level (85.7%) (Fall et al., 2015).

Specific aspects and constraints for MALDI-TOF MS in Africa

Constraints for acquisition and installation

The primary obstacle in performing microbial identification using MALDI-TOF MS is the cost of the equipment, which is estimated at between $120,000 and $270,000 (Tran et al., 2015). Electricity is another constraint,

as it must be supplied continuously for MS. Thus, the presence of an electric generator is required to prevent power failure. Moreover, the instrument, as well as all the connected computers, must be equipped with an inverter in case of micro-power cuts. The room in which the equipment is housed must be protected from insects and dust, and must be thermo-isolated; air conditioning is mandatory.

Constraints for routine microbial identification

The main reagent required to perform MALDI-TOF MS is the chemical matrix, which is not expensive, particularly when it is home-made (Seng et al., 2009; Martiny et al., 2014). Home-made solutions can also be freshly prepared each day in not more than 10 min and stored at room temperature for the day. None of the reagents (acetonitrile solution, water, trifluoroacetic acid solution, and α-Cyano-4-hydroxycinnamic acid) need to be frozen; α -Cyano-4-hydroxycinnamic acid is the only reagent that must be stored away from light. The chemical matrix must be stored at +4°C only when purchased matrices or home-made matrices prepared a few days before are used. Commercialized standards also need to be frozen at -20°C, but fresh *E. coli* cultures can also be used as standard. Thus, reagents are not a limitation to the process of microbial identification when home-made matrices are prepared on a daily basis and *E. coli* is freshly cultivated. Each system includes spot target plates, but the plates are reusable steel targets for the Microflex LT, while the VITEK® MS uses disposable plastic targets (Deak et al., 2015). Humans may be a constraint as staff must be previously and specifically trained in the use of MALDI-TOF MS. However, it is an easy system which does not require specific prior expertise. Moreover, the required skills are quickly acquired. For example, in Senegal, after a four-day course including theoretical and practical training, the four people who completed the training course provided by two engineers from bioMérieux were autonomous in the use of MALDI-TOF MS (Fall et al., 2015).

Constraints for maintenance

The second main obstacle to the use of MALDI-TOF MS in Africa is maintenance. Annual maintenance is recommended by the manufacturers, which raises two problems: its cost, including the cost of spare parts, labor and maintenance contracts; and the lack of trained personnel in Africa to perform it. The spare parts that need to be changed most frequently are the laser and detector (depending on frequency of use) and the primary pump (a lifespan of three to four years). Overall, for the MALDI-TOF mass spectrometer that was implanted in Senegal, when moderate problems are observed (two or three times per year), a web connection is established between the local instrument in Dakar and the company in France. This kind of maintenance concerns the fine tuning and the diagnostic of eventual issues. In parallel, maintenance is done once per year by moving an engineer from France.

Solutions

Funding for the acquisition and maintenance of MALDI-TOF MS in Africa is the main constraint for implementing the technology. Routine identification does not actually raise problems or limitations.

For this study, the cost of acquiring the apparatus in Dakar was covered and shared between several organizations, including the Institute of Research for Development, a public French organization involved in research with and for southern countries, the Mediterranean Infection Hospital-University Institute, which promotes the fight against infectious diseases on a global scale, and the French Ministry of Foreign Affairs (Fall et al., 2015; Lo et al., 2015). For others, research organizations, non-governmental organizations, or charity foundations, such as the Mérieux Foundation or the Melinda and Bill Gates Foundation, which are both already involved in the use of new tools to prevent and treat deadly diseases in Africa, could help fund this equipment.

Currently, the strategy applied in several countries to lower management costs involves grouping clinical microbiology laboratories into large core laboratories. Thus, the development of a common MS platform between several clinical microbiology laboratories in nearby areas would appear to be the best option to share the costs. The experience of MALDI-TOF MS networking in university hospitals in Belgium has recently been reported for identifying microorganisms in Brussels (Martiny et al., 2014).

Over a one-month period, 1,055 isolates were identified using conventional techniques from the first hospital and analyzed by MALDI-TOF MS in another hospital situated at 7.5 km away; target plates and identification projects were sent. Identification by the MALDI-TOF networking system was more accurate and faster than that carried out in parallel with conventional methods which led to a substantial annual cost savings (Martiny et al., 2014).

Twelve months ago, the study clinical microbiology laboratory (University Hospital, Marseille, France) also opened up access to MALDI-TOF MS platform for use by other hospitals: the public health hospital from Salon de Provence, a remote town 52 km away with 400 beds, and the military teaching hospital of Marseille (Laveran), a general hospital with open access for both military personnel and civilians with 303 beds (personal data). Every week, hundreds of bacteria were correctly identified at a low cost without moving patients. Thus, the

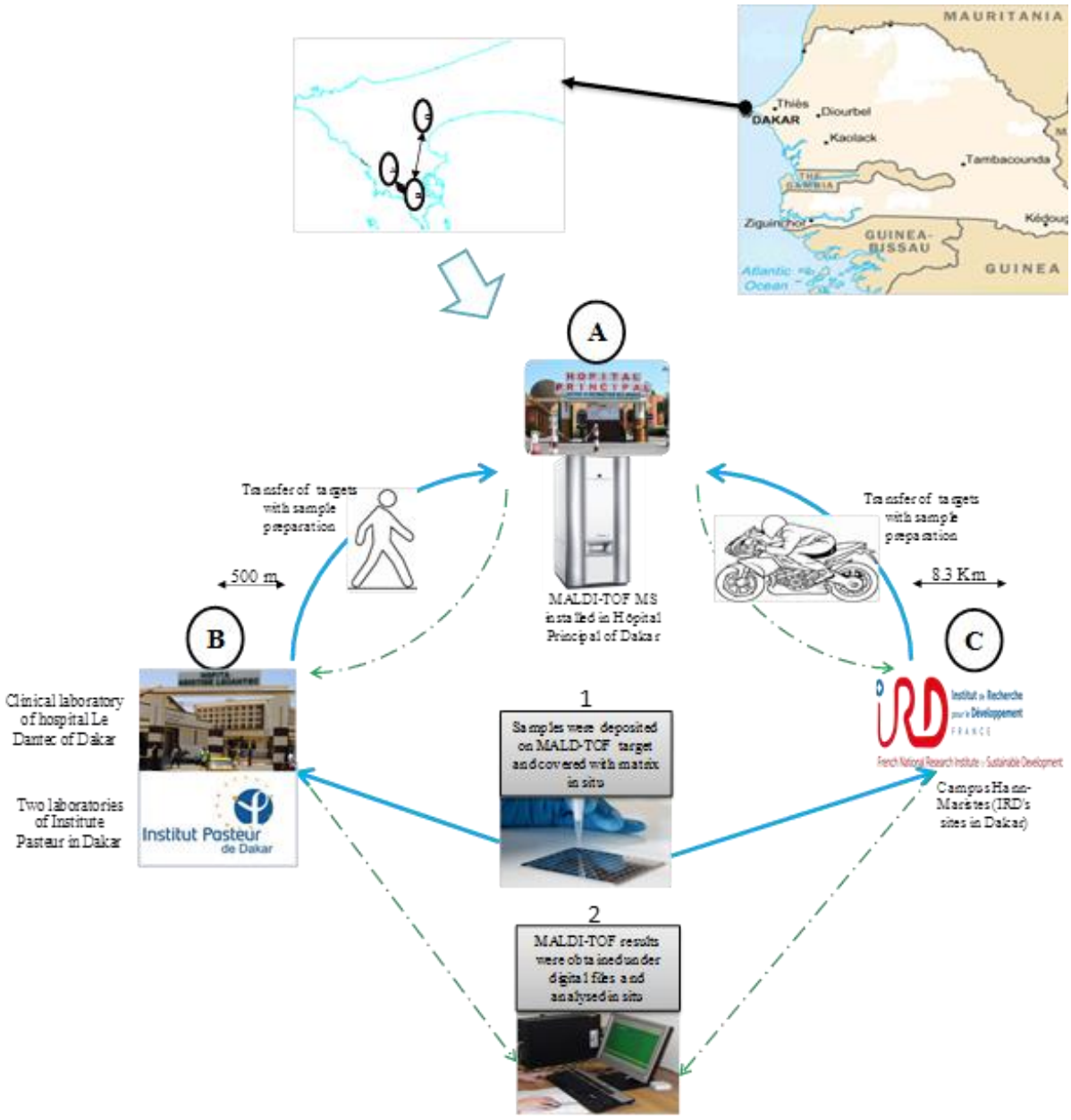

Figure 5. Schematization of the circuit for the use of a MALDI-TOF platform in Dakar (Senegal). MALDI-TOF MS is located in the clinical microbiology laboratory of Hôpital Principal de Dakar (A), but it is also used for diagnostic and research purposes by Hospital Le Dantec, the Institute Pasteur of Dakar (B), and by the Institut de Recherche pour le Développement (IRD) situated at the east of Dakar (C).

use of the same MALDI-TOF MS platform enables skills to be shared and reduces the cost of acquiring and maintaining the instrument (Martiny et al., 2014).

The MALDI-TOF mass spectrometer, that we managed, is installed at the Hôpital Principal de Dakar (Senegal) since 2012 (Fall et al., 2015). This platform is open to other health structures as well as research centers located in Dakar and its periphery as indicated on Figure 5. The samples shipment to the platform is frequent for centers like the Institute of Research for Development (IRD) and the Pasteur Institute but it is rarer for structures such as the Le Dantec Hospital and the public center of biologic and medical analysis of the Hôpital Abass Ndao.

Indeed, the platform becomes a real support for the identification of microorganisms isolated from patients in these structures. IRD prepares its own target plates; all the other structures send the strains they have been unable to correctly identify using conventional methods directly to the hospital. The time for target plate transfer

to the platform varies depending on the road traffic but it never exceeds an hour and a half. A low quality of deposit linked to transfer between sites and temperature has never been observed. When the target plates arrived at the platform, only the qualified personnel of the platform perform the plate's analysis. Interpretation of the data is also performed by the personnel of the platform, except for the plates from the IRD.

Indeed, qualified people and Saramis software (bioMérieux) are both available at the IRD. Thus, raw data can be retrieved and interpreted there. For other structures, interpreted data can be recuperated directly or send by email. If a MALDI-TOF MS platform is established, a cooperation agreement and a convention should be established between the various teams in order to specify not only the organization of workflows but also the tasks and responsibilities of everyone involved. Finally, local maintenance staff should be specifically trained.

CONCLUSION

The rapidity, efficiency, and low cost have led many laboratories to adopt the MALDI-TOF as a tool for routine diagnosis, resulting to an improvement in patient care. The first successful use of a MALDI-TOF mass spectrometer in Senegal supports the fact that it is a robust and potentially valuable tool in tropical Africa which should be widely distributed there. The development of shared MALDI-TOF MS platforms in nearby geographic areas will allow equipment, skills, and costs to be shared.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Isolation of bacteria from mobile phones before and after decontamination: Study carried out at King Abdulaziz University, Jeddah, Saudi Arabia

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Different fomites which are in regular contact with humans can play an important role in the transmission of microorganisms. Mobile phones have become indispensable in all walks of life; nevertheless their potential role in transmission of infections is of great interest. A cross-sectional study was done (April to June, 2015) at King Abdulaziz University, Jeddah, Faculty of Medicine (female campus), in order to detect the prevalence of bacterial contamination of mobile phones by students and staff, to investigate the most frequent habits associated with the use of mobile phones and effective cleaning of mobile phones with 70% alcohol for decontamination. A total of 168 swabs from 84 mobile phones derived from 80 volunteers were sampled at random. At the same time during sampling, a self-administered questionnaire was developed. All 84 mobile phones sampled were contaminated with bacteria, before decontamination. Coagulase-negative staphylococci were isolated frequently (32.3%), followed by *Staphylococcus aureus* (18.1%), viridans streptococci (15.7%), *Bacillus* spp. (13.4%) and *Corynebacterium* spp. (11.8%). Gram-negative bacilli and other Gram-positive cocci were also isolated but at lower levels. Mobile phones belonging to students had the highest rates of contamination (65.35%), followed by doctors (47%) and administrators (8.67%). Whilst, the lowest rate of bacterial contamination (5.5%) was observed among laboratory technicians, McNemar's analysis indicated that decontamination with 70% alcohol significantly decreased the rate of contamination from 100 to 47.6% (*P*<0.000). This study shows that all mobile phones examined were heavily contaminated with bacteria and the use of 70% alcohol for decontamination was effective in reducing bacterial colonization on these devices. Educating users on hygiene practices while using either mobile phones or other fomites in daily life aspects can help to reduce cross-transmission with microorganisms.

Key words: Medical campus, mobile phones, bacteria, decontaminations, hygiene, contamination, Saudi Arabia.

INTRODUCTION

Bacteria comprise an extremely diverse and widespread group of organisms, capable of inhabiting ubiquitous environmental niche. They grow rapidly as a result of their simple structure and genetic organization (Barer, 2002). Many have simple growth requirements and can withstand harsh environmental conditions, adapted to

growth on the skin of individuals (normal flora) and environmental surfaces. As consequence, there is a continuous exchange of flora between individuals and their environment (Engelkirk and Engelkirk, 2011).

Normal human bacterial flora was previously considered to be non-pathogenic and disregarded. In recent years their clinical importance as opportunist pathogens is increasing. These organisms can cause community and hospital-acquired infections, frequently producing disease when transferred from healthy individuals to susceptible hosts. Both direct and indirect contact has been implicated in such instances for a variety of different organisms (Soto et al., 2006; Arora et al., 2009; Elkholy and Ewees, 2010).

Indirect transmission via numerous objects such as objects, which have prolonged contact with the skin and those that are handled for extensive periods of time can transmit bacteria in health-care settings (Karabay et al., 2007; Kawo et al., 2009; Kilic et al., 2009; Ulger et al., 2009; Singh et al., 2010). Mobile phones (MPs) have recently become common in daily life aspects throughout the world, which require extensive human contact. Although, phones are important communication means in hospitals, their widespread use raise public health concerns as they may be implicated in the transmission of infections (Goldblatt et al., 2007; Karabay et al., 2007; Tagoe et al., 2011; Julian et al., 2012).

Several studies have shown that, MPs may be contaminated with pathogenic bacteria and serve as a vehicle for their transmission (Karabay et al., 2007; Tagoe et al., 2011). Further, it has been established that contamination of various other user interfaces can differ geographically and also within different institutions or communities (Oluduro et al., 2011). This is probably associated with variation in usage habits and implementing of hygiene practices.

Literature on bacterial contamination of MPs in the Kingdom of Saudi Arabia (KSA) till date remains scanty (Al-Abdalall, 2010; Zakai et al., 2016). Therefore, this study was initiated in order to establish the importance of MPs as possible vehicles for infectious bacteria, amongst preclinical female students and staff at a medical campus in KSA to investigate the effective cleaning of mobile phones with 70% alcohol for decontamination.

MATERIALS AND METHODS

Sampling

A cross-sectional study was conducted (April to June, 2015) at the King Abdulaziz University (KAU), Faculty of Medicine (female campus). Two samples were collected from each mobile phone. The first swab was taken before decontamination and the second after cleaning the device with 70% alcohol wipe. A total of 168 swabs from 84 MPs derived from 80 volunteers (4 participating staff had two MPs) were randomly sampled. The study groups included; 52 preclinical medical students (2nd and 3rd year), 8 nursing students, 11 doctors, 5 laboratory technicians and 4 administrative staff.

The samples were taken with a sterile cotton swab which was moistened with sterile saline solution and the target phone was wiped off the surface on both sides of the mobile (that is over the keypad and back of the mobile phones, in case of mobile phones with covers, swab taken from the outer surfaces of the cover) using aseptic techniques (sterile gloves were used to avoid cross-contamination).

Data collection

During sample collection, a self-administered questionnaire was used to collect information regarding the socio-demographic data relating to the nature and frequency of use, cleaning habits, contact with animals, use in toilets and regular sharing of their MPs. Written informed consents for participation were obtained from all of the volunteers who allowed us to collect samples from MPs for this study.

Bacteriological analysis

Samples were inoculated immediately and aseptically into 10 ml of brain-heart infusion broth (Saudi Prepared Media Laboratory, SPML, Riyadh) and shaken using a vortex mixer for 1 min. All tubes were labeled with specimen number and incubated at 37°C for 24 h aerobically. After incubation, specimens were sub-cultured on 5% sheep blood (SPML, Riyadh) and MacConkey (SPML, Riyadh) agar plates at 37°C for 24 to 48 h. After this, plates were examined for growth and colonial morphology of the isolates.

Preliminary identification of bacteria was based on Gram reaction, colony characteristics (colonial morphology), and change in physical appearance of different media, and presence of catalase and oxidase (Oxoid Limited, UK) enzymes. Gram-positive, catalase-positive cocci were tested for mannitol fermentation and coagulase development by using mannitol salt agar (SPML, Riyadh) and coagulase test (Oxoid Limited, UK), respectively.

Antimicrobial susceptibility testing

All *Staphylococcus aureus* (*S. aureus*) and enterococci strains were screened for oxacillin (1 µg) (Oxoid Limited, UK) and vancomycin (30 µg) (Oxoid Limited, UK) resistance using the Kirby-Bauer disk diffusion method on Muller-Hinton agar (SPML, Riyadh), according to the Clinical and Laboratory Standards Institute Guidelines criteria (CLSI, 2012).

The test strain was suspended in a nutrient broth (SPML, Riyadh) and incubated for 30 min to make it comparable with 0.5%

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McFarland standard. After incubation, a sterile cotton swab was dipped into suspension and bacteria were inoculated on Muller-Hinton agar (SPML, Riyadh). Antibiotic discs were placed by using a disc dispenser and plates were incubated for 24 h at 37°C.

Statistical analysis

Data was entered and analyzed using Statistical Package for Social Science (SPSS, version 21). Comparisons of data were performed using McNemar's test; *P*-value of less than 0.05 was considered as statistically significant. Statistical analysis was carried out in Statistics and Information Processing Unit, King Fahad Medical Research Center, in KAU, Jeddah, Saudi Arabia.

RESULTS

Epidemiological survey

Demographic results showed that the age range of the participants was between 19 to 55 years; all were female. About 70% of participants used "iPhones" rather than other smart phones and 83.75% used their phones extensively (several hours). Almost half of those surveyed (57.5%) used their phones for both work and personal use. The most common use for smart phone was sending or receiving texts and making or receiving calls.

In addition, individuals indicated using social networking websites, internet, e-mail for studying and downloading lectures. Few reported use of their device to take, send and receive photos or to record, send, receive and play videos. Many participants indicated that in the absence of a watch, they use their phones to keep time.

About 8.75% reported that they never decontaminated their devices; while about half of the participants (52.5%) decontaminated their phones when visibly dirty. Others clean their phones at different intervals. About 11.25% of participants decontaminated their mobile with alcohol wipe and 33.75% stated they just wiped with their sleeve or cloth. About 47.5% reported that they used their phones in toilet and 93.75% indicated they washed their hands after using toilet. About 78.75% of participants had no pets whilst, 26.25% of those responding stated they share their cell phone with friends or family members.

Overall bacteriological results before decontamination of mobile phones

All 84 phones screened in this study showed bacterial growth before decontamination. The findings indicated that coagulase-negative staphylococci (CNS) (n=41), *S. aureus* (n=23), viridans streptococci (n=20), *Bacillus* spp. (n=17), *Corynebacterium* spp. (n=15), Gram-negative bacilli (GNB) (n=8), *Enterococcus* spp. (n=2) and *Micrococcus* sp. (n=1) were most frequently associated with mobile phones. The recovery rate was between 0.8 and 32.3% (Table 1).

Distribution of results according to occupation

Mobile phones belonging to students had the largest variety of bacteria with highest rates of contamination (83/127; 65.35%), followed by doctors (26/127; 20.47%) and administrators (11/127; 8.67%) whilst, the lowest rate of bacterial contamination (7/127; 5.5%) was observed among laboratory technicians.

Pathogenic bacteria such as *S. aureus* were mostly isolated from MPs of medical students (19/23; 82.6%) (Table 2). Neither methicillin-resistant *Staphylococcus aureus* (MRSA) nor vancomycin-resistant enterococci (VRE) were isolated from MPs in this study.

Overall bacteriological results after decontamination of mobile phones

All 84 MPs sampled in this study were contaminated with bacteria before decontamination, and this made an isolation rate of 100%. When the rate of bacterial isolation was evaluated after decontamination with 70% alcohol from the MPs, assessed growth was observed in 40 (47.6%) and about 44 (52.4%) of mobile phones did not show any growth after decontamination (Table 3).

Figure 1 shows type and frequency of bacteria isolated from MPs of medical students and staff in Faulty of Medicine (female campus) at KAU before and after decontamination with 70% alcohol.

In this study, 70% alcohol showed a significant reduction in the rate of mobile phone contamination with McNemar's test (*P*-values) of 42.023 (<0.000).

DISCUSSION

Mobile phones due to their personal nature and proximity to delicate parts of our bodies in usage such as faces, ears, lips and hands of users could become important modes of transmission for pathogens that could result in infections (Karabay et al., 2007; Kilic et al., 2009).

Bacterial contamination was found on all MPs investigated in this report. Other workers in Nigeria (Ilusanya et al., 2012), Ghana (Tagoe et al., 2011), Egypt (Selim and Abaza, 2015) and in India (Kumar and Aswathy, 2014) have also similarly reported contamination on all devices investigated. Reporters have documented varying levels of contamination ranging from 43.6 to 98% (Akinyemi et al., 2009; Sadat-Ali et al.,

Table 1. Bacteria isolated from mobile phones.

Bacterial isolate	Number recovered	Percentage
Coagulase-negative staphylococci (CNS)	41	32.3
Staphylococcus aureus	23	18.1
Viridans streptococci	20	15.7
Bacillus spp.	17	13.4
Corynebacterium spp.	15	11.8
Gram-negative bacilli (GNB)	8	6.3
Enterococcus spp	2	1.6
Micrococcus sp.	1	0.8
Total	127	100

More than one type of bacterial growth was seen in some mobile phones.

Table 2. Distribution of bacteria isolates from mobile phones according to occupation.

Bacterial isolate	Students (n=60)	Doctors (n=11)	Lab technicians (n=5)	Administrator (n=4)
Coagulase-negative staphylococci (CNS)	26	9	3	3
Staphylococcus aureus	19	2	2	-
Viridans streptococci	12	3	1	4
Bacillus spp.	10	4	1	2
Corynebacterium spp.	11	2	-	2
Gram-negative bacilli (GNB)	3	5	-	-
Enterococcus spp.	1	1	-	-
Micrococcus sp.	1	-	-	-
Total	83	26	7	11

Table 3. Bacterial growth after decontamination of mobile phones with 70% alcohol.

Cell phone	Growth positive	Growth negative	Total	*P* value
Before	84	0	84	42. 023
After	40	44	84	

2010; Elmanama et al., 2015; Roy et al., 2013; Gashaw et al., 2014). These differences in microbial contamination can be attributed to varied usage habits and frequency of cleaning the device (Ovaca et al., 2012; Mark et al., 2014).

This study showed that participants were using their MPs extensively each day, with majority (83.75%), stating that their daily usage totaled at many hours each day. This is much higher than a previous report stating that 25% of the participants did not use their device at work and a further 52% used their phones on less than 10 occasions in a day (Mark et al., 2014). These high contamination rates can be attributed to several factors; such as extensive use (many hours each day), frequent use in toilets as reported by almost half of the volunteers and sharing the device with family or friends also reported in about a quarter of those investigated.

A previous investigation in Kuwait documented that 33.5% of their participants “Never” cleaned their MPs and 73% of these devices yielded microbial growth (Heyba et al., 2015) whilst other workers from Egypt reported that 96.5% of those surveyed “Never” cleaned their device and 92.5% of those MPs were contaminated (El-Ashry and El-Sheshtawy, 2015). During the course of this work, 8.75% of those volunteering stated they “Never” cleaned their device. For the majority of our volunteers however, cleaning constituted simply wiping their MPs on their clothes and sleeves. The use of alcohol wipe for cleaning

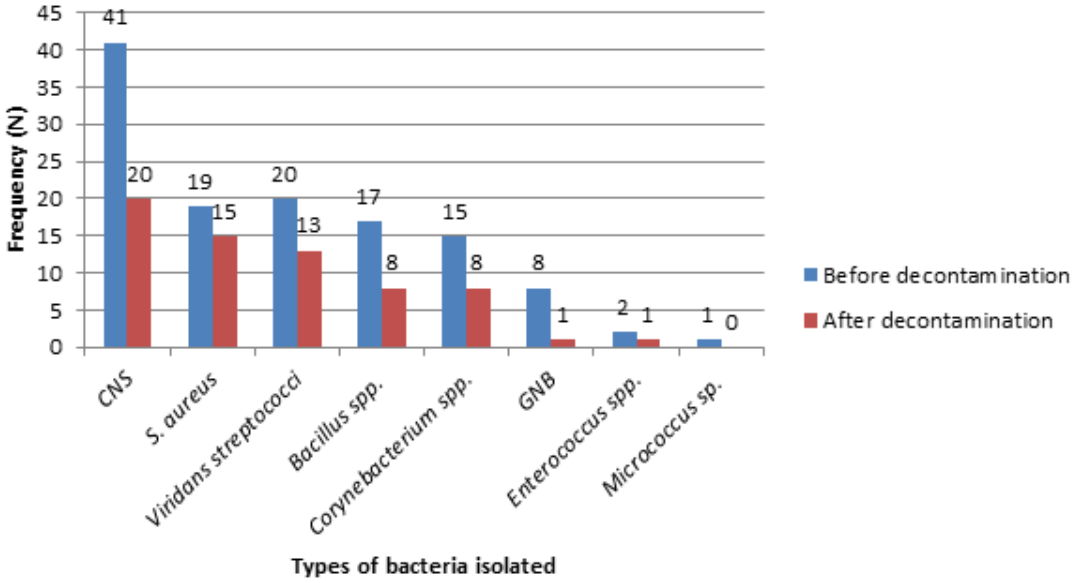

Figure 1. Type and frequency of bacteria isolated from mobile phones before and after decontamination.

was reported by only 11.25% of our subjects. It is possible to speculate that dry-wiping methods (on clothing) do not reduce microbial contamination of the MPs which may even enhance it.

Gram-positive bacteria were isolated more often (93.7%) than Gram-negative bacteria (6.3%) in this study. Similar results have been reported previously (Arora et al., 2009; Zakai et al., 2016). Most normal microbial skin flora, are Gram-positive bacteria (Roth and James, 1998), a fact that explains their predominance on MPs. Coagulase-negative staphylococci (32%), *S. aureus* (18.1%), viridans streptococci (15.7%) *Bacillus* spp. (13.4%) and *Corynebacterium* spp. (11.8%) were the most frequently isolated organisms in this experience; these findings are in line with other reports (Akinyemi et al., 2009; Arora et al., 2009; Roy et al., 2013; Heyba et al., 2015; Zakai et al., 2016).

In addition, it has been previously established that frequent contact with skin microbial flora and sustainable temperatures for bacterial growth attained on the device while in use provide a favorable environment for the growth of these microorganisms (Roth and James, 1998). Storage of MPs in pockets, handbags and brief-cases is likely to further encourage bacterial growth with warm and protected surroundings environment (Brady et al., 2006).

In this study, CNS (32%) was the most prevalent species. Other research has similarly shown CNS often, ranging from 22 to 68% of all isolates (Roy et al., 2013; El-Ashry and El-Sheshtawy, 2015; Heyba et al., 2015; Zakai et al., 2016). Although non-pathogenic in normal circumstances, their presence in high numbers on objects involving frequent hand contact like MPs in settings like intensive care units (ICUs) may pose a risk of bacteremia in immunocompromised patients (Brady et al., 2006). In addition, diseases such as late-onset neonatal sepsis, endophthalmitis and urinary tract infections have been documented (Rogers et al., 2009; Sgro et al., 2011). *S. aureus* isolated from 18.1% of MPs investigated are recognized pathogens, frequently isolated as the causative agents from a wide range of infections, ranging from simple skin infections (pimples and boils) to serious life threatening pneumonias and meningitis (Engelkirk and Engelkirk, 2011). The presence of *S. aureus* on MPs as on other fomites in hospital settings therefore is of significant concern. Our results show that *S. aureus* were mostly isolated from MPs of medical students (19; 82.6%). Other studies have also concluded that *S. aureus* was detected very often (Akinyemi et al., 2009; Ilusanya et al., 2012; Zakai et al., 2016). *S. aureus* is a major component among the normal flora of the skin and nostrils. Its predominance in the bacterial contaminants on MPs may be because it is easily discharged by numerous human activities such as sneezing, coughing, talking and other actions involving skin contact (Itah and Ben, 2004)

Bacillus spp. was commonly associated with MPs in this study (13.4%). This finding is explained by the ubiquitous nature of this organism in the environment, since it can survive harsh conditions being one of the few organisms that can sporulate and spread when met with sub-optimal conditions (Brooks et al., 2013). *Bacillus* spp.

like CNS although generally considered to be of low virulence are known to be opportunist pathogens in patients predisposed to infections.

By contrast, a previous report from KSA has documented higher levels of GNB than our current findings (Al-Abdalall, 2010). Presence of GNB in high proportions suggests contamination of MPs with faecal flora; such organisms often originate from soil, clothing, food and/or on the hands of the users (Al-Abdalall, 2010) can result in community-acquired infections. When GNB are detected frequently in fomites, special attention is advocated to hand washing and hygienic practices. In conjunction with this view, the use of the MPs in toilet facilities has also been a subject of some interest. In the present study, 47.5% reported the use of MPs in toilets, while other workers have documented 59% of their participants (Zakai et al., 2016). However, encouragingly regular hand washing practice was reported by 93.75% of our subjects but was not investigated in the previous study (Zakai et al., 2016). Similarly, the efficacy of good hand hygiene in reducing contamination of MPs was reported previously (Goldblatt et al., 2007). This seems to be corroborated by the finding that we did not isolate faecal flora on the MPs which were investigated very often. Hence, the importance of hand washing can be emphasized further.

Neither MRSA nor VRE were isolated from MPs in this study. These findings corroborate other reports both from Saudi Arabia (Al-Abdalall, 2010) and some other countries (Akinyemi et al., 2009; Singh et al., 2010). However, other investigators have previously detected MRSA from MPs, this may have been a reflection of the fact that these reports originated entirely from healthcare settings (Julian et al., 2012) and the higher prevalence of MRSA generally in hospital settings is well known (Barer, 2002; Ulger et al., 2009).

Mobile phones belonging to students had the greatest variety of bacteria with highest rates of contamination (83; 65.35%), followed by doctors (26; 20.47%) and administrators (11; 8.67%) whilst, the lowest rate of bacterial contamination (7; 5.5%) was observed among laboratory technicians. The level of contamination varied among different occupational groups; as this could be related to differences in hygiene level and sanitary practices together with education, extent of mobile use, environmental pollution and sharing mobile phone with friends or family members (Rusin et al., 2002; Akinyemi et al., 2009; Kawo and Musa, 2013). The variation in sample size could have been the reason for this observation.

It is possible to suggest that students generally spend more time handling their devices for different purposes (including time consuming research tasks) and may have a tendency to pass their MPs between friends, to share information with lack of professional recommendations on how to clean the mobile phones to meet hygiene standards. Laboratory workers on the other hand due to the nature of their work are unlikely to handle MPs for extended periods of time during working hours and are trained to wash their hands more frequently following their laboratory work. Most laboratories have posters informing staff about the importance of hand washing in designated areas and have facilities for washing within the laboratory area.

A high rate of bacterial contamination of mobile phones (100%) was recorded in this study before decontamination. The efficacy of decontamination with 70% alcohol at 52.4% was statistically significant (McNemar's test =42.023; *P*-value <0.000). This suggests that the use of a decontaminating agent such as 70% alcohol, play an important role in reducing bacterial colonization on MPs. Other workers have reported the efficacy of 70% alcohol in decontaminating MPs at 47% (Gashaw et al., 2014) and 98% (Arora et al., 2009).

Previous work has shown that the use of “antibacterial putty” is effective in reducing microbial contamination of phones (Ovaca et al., 2012). It is pertinent to suggest that in hospital settings such antibacterial agents or stronger ones are required to effectively decontaminate MPs. Other investigations have shown that implementation of regular cleaning with isopropyl alcohol wipes or 0.5% chlorohexidine and 70% isopropyl alcohol successfully reduced contamination rates of MPs (Beer et al., 2006; Goldblatt et al., 2007; Jayalakshmi et al., 2008).

Further work using different agents for decontamination of MPs is required in order to recommend standard cleaning practices especially in hospital settings. Hospital Infection Control Units must review policies regarding the use of MPs by personnel in patient contact and draw up suitable guidelines for use, stipulating cleaning policies and periodic sampling of MPs, in order to differentiate between transient and resident MPs flora. We envisage that guidelines such as those presently in existence for hand washing and food handlers are warranted for MPs. In addition, further research into the materials used for MPs manufacture with a view of finding those which discourage bacterial growth may be relevant. The use of “disposable MPs covers” in hospital settings in areas where other infection control methods such as gloves, aprons and masks are usually required may be stipulated.

Conclusion

Results in this study shows that all MPs samples carried numerous bacteria, of which some are known as opportunist pathogens. Application of 70% alcohol which resulted in significant reduction of bacterial contamination, suggest a potential decontaminating agent for MPs. In

addition, it is an easily accessible, safe and cheap agent.

Other factors such as personal hygiene and hand washing in particular are instrumental in preventing the transfer of microorganism from user hands to fomites such as MPs and vice versa. These findings substantiate the need for future investigations in order to monitor the transfer of pathogenic bacteria mediated by MPs and to educate users on the potential health-risk that may be posed by contaminated fomites such as transmission of infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Challenges in diagnosis of central nervous system infections using conventional method: Need for better approach in Rwanda

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Central nervous system (CNS) infection is a common and serious disease that needs rapid and appropriate diagnosis for an appropriate treatment. However, in most recourse limited setting including Rwanda, conventional microbiological method is the only way to establish a confirmed infectious etiology. This was a 4 years retrospective review of registers and electronic laboratory records aimed to determine the causative agents in hospitalized patient's suspected to be suffering from CNS infection at four referral hospitals in Rwanda. In this review, the majority of participants (48%) were in the age group between 25 and 44 years (median = 34), with 53 and 47% being males and female, respectively. Cerebrospinal fluid (CSF) was clear in 67% (112/168), turbid in 30% (50/168) and bloody in 3% (6/168) patients. Only 1% (2/168) of the samples had WBC count >1,000 cells/mm^3 and 13% (21/168) had WBC count between 101 and 1,000 cells/mm^3, WBC count between 10 and 100 cells/mm^3 was present in 10% (17/168) whereas <10 cells/mm^3 was present in 76% of the samples. The present data above was from one selected referral hospital out of four sites used in this study, whereby all required data were funded during data collection. Out of 208, positive CSF was identified from four sites; *C. neoformans* was the most frequent pathogen isolated, followed by *Streptococcus pneumoniae* representing 71.6 and 9.6%, respectively. Other pathogens identified included *Acinetobacter* spp. which represented 4.3%, *S. aureus* 3.8%, *E. coli* 2.8% and *K. pneumonia* 1.9%. Both *N. meningitides* and *H. influenzae* type B were isolated in only 0.48% for each. The present study reveals that the diagnostic of CSF infection using conventional method is alarmingly low across all tertiary hospitals, suggesting further studies using molecular methods to shed light on the etiological agent of CNS infections in Rwanda.

Key words: Central nervous system (CNS) infection, conventional method, causative agents, diagnostic capacity and resource poor setting.

INTRODUCTION

Meningoencephalitis is a disease characterized by, inflammation of the meninges and brain tissue due to infection by microorganisms such as bacteria, virus, parasites and fungi (Shaban and Siam, 2009). In the clinical setting, this diagnosis is often considered in any patient presenting with fever, headache and/ or altered mental status who happens to be found with meningeal irritation signs on physical examination (Fouad et al., 2014).

In infected individuals with encephalitis, symptoms such as headache, fever, vomiting, confusion and light-sensitiveness are common and can in severe cases also cause unconsciousness, seizures and paralysis. Viral meningoencephalitis is the most common but least severe form with almost all patients recovering without any permanent pathological changes, although full recovery might sometimes take weeks.

Bacterial meningoencephalitis is often more severe than viral meningoencephalitis and can lead to permanent pathological changes or death in around 50% of untreated cases, and accounts for around 170,000 deaths globally each year (Boving et al., 2009). Most cases of bacterial meningoencephalitis are caused by *Streptococcus pneumonia* (Afifi et al., 2007). *Streptococcus agalactiae, Neisseria meningitidis, Listeria monocytogenes* and *Hemophilus influenza B* are examples of bacterial meningoencephalitis (Akhvlediani et al., 2014). Bacterial meningitis reaches the sub-arachnoid space by hematogenous route or may directly reach the meninges in patients with parameningeal focus of infection. Bacterial meningitis usually can cause brain damage, hearing loss, limb amputation, learning disabilities and even death (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007).

Fungi cause severe infections but are much less frequent than bacterial or viral infections (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007). The most common causes of fungal meningoencephalitis are *Cryptococcus neoformans, Candida albicans* and *Aspergillus* species mainly in immune-compromised patients (Baskin and Hedlund, 2007). *C. neoformans* is an encapsulated basiodiomycetes fungus of medical importance, capable of crossing the blood brain barrier and causing meningitis in both immunocompetent and immunocompromised individuals.

The best characterized virulence factors for *C. neoformans* include the production of polysaccharide capsule, the formation of melanin and the ability to grow at 37°C which is an essential virulence factor for pathogenesis. Polysaccharide productions within phagocytic cells contribute to fungal survival (Steen et al., 2003). The main symptoms of fungal meningoencephalitis are fever, vomiting, headache, stiff neck, sensitivity to light and drowsiness. The serious and disabling complications include hearing loss, brain damage or learning difficulties (Baskin and Hedlund, 2007; Minjolle et al., 2002; Růzek et al., 2007).

In order to minimize unnecessary antibiotics, antiviral and antifungal prescriptions and to determine the appropriate treatment, it is important to identify at an early stage whether meningitis, encephalitis or meningoencephalitis is caused by bacteria, viral or fungal pathogens (Baskin and Hedlund, 2007). This also would minimize the exposure of patients to side effects of medications they do not require. The current treatment guidelines for the treatment of meningoencephalitis in developing countries are based on data obtained from countries with robust economies and strong immunization programs.

This might be misleading in resource-limited settings, which may have completely different disease aetiologies. Thus, there is a need for descriptive epidemiology of infective agents of meningoencephalitis in different geographical locations, as the pathogens vary depends on environmental circumstances, amongst other things.

Currently in Rwanda, conformation of infectious aetiology in patients with acute Central nervous system (CNS) infections completely relies on conventional diagnostic methods, consisting of routine cultures. The availability of reliable and improved diagnostic tools such as molecular method is extremely limited. The current study was undertaken to examine the utility of conventional microbiological methods in the identification of the causative agents in hospitalized patients, suspected of cerebrospinal fluid (CSF) infection. It also aimed to provide useful baseline data, highlighting the major gap in the diagnostic capacity for CNS infection in Rwanda.

MATERIALS AND METHODS

All patients meeting the inclusion criteria and willing to participate in

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the study as indicated by signing an informed consent were included in the study. For each patient, laboratory registers were accessed to extract information relevant to this study. This consisted of descriptive retrospective hospital record results from analyzed CSF samples. Using conventional microbiological methods, the present hospital based study findings were drawn from 2,410 hospitalized patients with suspected CNS infections admitted in 4 referral hospitals in Rwanda.

Complimentary data was obtained using electronic laboratory records by, retrieving retrospectively hospital record results from analyzed CSF samples. All data was captured from analyzed CSF samples results as evidence of meningoencephalitis or absence of causative agents. The data was recorded in Excel spreadsheets and was transferred to SPSS for further analysis.

CSF samples collection and transport

Prior to data collection, the principal investigator held a meeting with all the experienced staff in cerebrospinal fluid (CSF) collection in all the research sites, to discuss sample collection and transportation to a central laboratory. The CSF samples were obtained using standard guidelines for sterile clinical procedures. In brief, before lumbar puncture procedure, the patients were made to lie down at an appropriate position, the skin was disinfected along a line drawn between the crests of the two ilia with 70% alcohol and iodine and allowed to dry completely.

The clinician then injected the spinal needle into the skin between the 4 and 5th lumbar vertebral spines with the bevel of the needle facing up. As soon as the needle was in position, the CSF pressure was measured and a sample of 3 to 4 ml of the fluid was collected in two sterile screw tubes for testing, using conventional and molecular diagnostic methods.

Collection specimen

At all sites, the obtained CSF were collected prior to antimicrobial therapy and placed into at least 3 separate sterile leak proof tubes before being transported to the laboratory. One tube from the collected CSF specimens was transported to bacteriology laboratory immediately without refrigeration. The CSF was processed in a biological safety cabinet to avoid contamination of the specimen and/or the primary inoculation medium. Blood agar plate, chocolate agar, thioglycolate broth and Sabouraud agar were used for the culture of specimens.

Processing of CSF and gram staining

Initial processing of CSF started by recording the volume of CSF and its gross appearances namely clear, bloody, cloudy, or xanthochromic. Clear CSF samples were tested using cryptococcal latex agglutination and India ink microscopy for detection of cryptococcal neoformans antigen and yeast cells surrounded by a characteristic polysaccharide capsule, respectively. Adequate turbid samples were tested using latex agglutination for detection of specific polysaccharide surface antigens, for most common bacteria as causative agents of meningitis. The specimen was then centrifuged for 20 min at 1,500 to 3,000 ×*g* if the volume recorded was >1 ml. The sediment was vortexed vigorously for at least 30 s to resuspend the pellet.

Using a sterile pipette, media was inoculated by placing 1 or 2 drops of sediment on an alcohol-rinsed slide, allowing drop to form a large heap. The slide was then air dried on a slide warmer before being gram stained as described by Fouad et al. (2014). The CSF gram stained smears were examined and interpreted immediately and all positive smears were immediately reported to the physician and nursing unit by telephone. The telephone notification was documented.

Culture examination

All collected CSF samples in four referral hospitals were transported within 1 h and centrifuged at 1000 ×*g* for 10 to 15 min with supernatant, used for rapid diagnostic test. Sediment was used for gram stain and primary plating on chocolate, blood agar, MacConkey agar and sabouraud dextrose agar.

All plated and thioglycolate broth media were examined for macroscopic evidence of growth. With no visible growth on the culture media, broth was re-incubated and negative plates were examined daily for 72 h before discarding. Broth media was also examined daily for 5 to 7 days before discarding.

Culture with growth and organism identification

From colony appearance, colony was picked to prepare gram stain broth, if positive. Semi quantitative growth was put on plated media and gram stain prepared for each morphotype. The microorganisms were identified based on the morphology of colonies, and by biochemical reaction and serotyping.

Antimicrobial sensitivity was also done according to the isolated microorganisms. The physician was notified of culture findings and antibiotic sensitivity patterns.

Ethical considerations

Before any data collection, ethical approval to conduct the study was obtained from Rwanda National Ethical Committee (No.472/RNEC/2009) and respective hospital ethical committees. Privacy and confidentiality of the patients were upheld at all times. There were no personal identifiers in all the samples obtained and instead, a unique number was used for each sample.

RESULTS

As shown in Table 1, the majority of participants (48%) were in the age group between 25 and 44 years (median = 34), with 53 and 47% being males and female, respectively. Macroscopic appearances and cytological (cell count) findings of CSF samples in patients with clinically diagnosed central nervous system infections were available and complete at one site of the study as described in Tables 2 and 3, respectively. CSF was clear in 67% (112/168) patients and turbid in 30% (50/168) patients. About one-quarter, that is 3% (6/168) patients had their CSF contaminated with blood.

Table 1. Sample descriptions of the study by age group and gender.

Age group (median: 34 years)	Gender		Total (%)
	F	M	
<5	4	9	13 (8)
5-14	8	8	16 (10)
15-24	12	3	15 (9)
25-34	25	19	44 (26)
35-44	11	25	36 (21)
45-54	11	17	28 (17)
>55	8	8	16 (10)
Total	79	89	168 (100)

Table 2. Macroscopic appearance of CSF samples in CSF culture in patients with clinically diagnosed central nervous system infections.

CSF appearance	CSF culture	
	Frequency	Percent
Clear	112	67
Turbid	50	30
Bloody	6	3
Total	168	100

Table 3. CSF WBC count distribution according to CSF culture in patients with clinically diagnosed CNS infections.

WBC count in CSF	CSF culture	
	Frequency	Percent
<10	128	76
10-100	17	10
101-1000	21	13
>1000	2	1
Total	168	100

There was no difference in culture positivity rate (33 vs. 28%) in patients with turbid CSF compared to patients with clear CSF. Blood stained CSF had a culture yield of 17%. CSF cytology revealed that only 1% (2/168) of the samples had WBC count >1,000 cells/mm^3, 13% (21/168) had WBC count between 101 to 1,000 cells/mm^3, WBC count between 10 and 100 cells/mm^3 was present in 10% (17/168) whereas <10 cells/mm^3 was present in 76% of the samples. The presented data above was from one selected referral hospital out of four sites used in this study whereby all required data were funded during data collection.

The patients with elevated CSF (>1,000 WBC/mm^3) WBC count had a culture positivity rate of 55.5%. Distribution of laboratory culture findings is shown in Table 4. *C. neoformans* was the most frequent pathogen isolated followed by *S. pneumonia,* representing 71.6 and 9.6% of isolates, respectively. Other pathogens isolated included *Acinetobacter* spp (4.3%), *S. aureus* (3.8%), E*. coli* (2.8%) and *K. pneumonia* (1.9%). Both *N. meningitides* and *H. influenzae* type B were isolated in 0.5% each.

Table 4. Distribution of laboratory culture results of 208 positive CSF among 2,410 hospitalized patients with clinically diagnosed CNS infections.

Pathogen	2009		2010		2011		2012		Total
Pathogens isolated fungi	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**	**Percent (%)**	**Frequency**
C. neoformans	34	67	41	73	36	69	38	78	149
Pathogens isolated bacteria									
S. pneumoniae	7	14	5	9	2	4	6	12	20
N. meningitidis	0	0	0	0	1	2	0	0	1
H. influenzae type B	0	0	0	0	1	2	0	0	1
S. aureus	4	8	1	2	3	6	0	0	8
Acinetobacter spp.	3	6	1	2	3	6	2	4	9
E. coli	0	0	2	4	2	4	2	4	6
K. pneumoniae	0	0	0	0	3	6	1	2	4
Others*	**3**	**6**	**6**	**11**	**1**	**2**	**0**	**0**	**10**
Total	**51**	**-**	**56**	**-**	**52**	**-**	**49**	**-**	**208**

DISCUSSION

Central nervous system (CNS) infection is a common and serious disease that needs rapid and appropriate diagnosis for an accurate treatment. The availability of reliable and improved diagnostic tools such as molecular method is extremely limited in resource-constrained countries such as Rwanda, and therefore conventional microbiological method is the only way to establish a confirmed infectious etiology in patients with acute CNS infections.

In the present study, demographic data review showed that majority of participants (48%) were in the group age between 25 and 44 years (mean = 34), with 53% being males; these findings are consistent with those of Fouad et al. (2014), who confirmed that males were more significantly affected with bacterial meningitis than females; 61 versus 39%, respectively. The majority of the patients in the present study were middle aged as has previously been described. A study by Fouad et al. (2014) found that patients with meningoencephalitis were middle aged with a mean age of 38.3 year. The reason for this age group being most affected is not known.

The low yield of microorganisms from CSF demonstrated in this 4-year retrospective review is similar to that reported in earlier studies (Becerra et al., 2013). For instance, our finding of 8.6 bacterial culture yield is comparable with studies in developing countries which reported low rates of culture positive CSF samples ranging from 8 to 10% (Wu et al., 2013) but higher than a study finding in Georgia (Khater and Elabd, 2016) which reported a positivity rate of 3.6%. There are several reasons why the diagnostic yield may be low when conventional microbiological methods are employed to identify etiological causes of CNS infections.

A potential reason for these results may be the known poor performance of conventional microbiological methods in the etiological confirmation of causative microorganism in CNS infections. Other possible reasons reported elsewhere indicate that, low diagnostic yield of conventional microbiological methods may be largely affected by the antibiotic use prior to

hospital admission. In fact, a culture positivity rate of 10% has been reported in patients previously treated with antibiotics in developing countries (Wu et al., 2013). Although information on prior antibiotic use was not recorded, previous use of antibiotics may have been higher in our study population, because the majority of these patients had been treated in lower level health facilities before being referred and admitted to a referral health facility.

Additionally, the observed low diagnostic yield could also suggest that other causative agents whose identification requires more improved methods are not used in this group of patients such as *Mycobacterium tuberculosis* or viruses where pathogen is involved. This is not surprising as in a previous study, the use of molecular diagnostics was particularly important in identifying a pathogen in 90% of culture-negative cases (Khater and Elabd, 2016). Taken together, our findings with these demonstrate the big challenge associated with the identification causative agents in CNS infections, in settings with limited diagnostic capacity like Rwanda. It also supports the hypothesis that, the use of molecular methods could improve pathogen identification for CNS infections. These emerges from this study that, the most common microbial pathogen causing meningitis in all the four referral care hospitals is *C. neoformans,* which account for 71.6% in all cases.

These findings are in line with previous studies in the region and often correlate with the high causative agents in CNS infections in settings with limited diagnostic capacity like Rwanda. It also supports the hypothesis that the use of molecular methods could improve pathogen identification for CNS infections (McCarthy et al., 2006). However, in our study this observation may be due to the fact that the diagnosis of this fungus is very simple and readily available in all hospitals included in our study.

On the other hand, *S. pneumonia* represents the second most common pathogens (9.6%) and was reported as the most common bacteria (33%) isolated in their studies (Campagne et al., 1999). It is evident that pneumococcal meningitis is currently the leading cause of bacterial meningitis in Rwanda as opposed to few cases (1/59) of meningococcal and *H. influenza* type b (1/59) meningitis, which indicate the increased use of both polysaccharide meningococcal and *H. influenza* type b vaccines in routine in Rwanda. Although isolated in small number (20/59), our study revealed no difference in the trend of *S. pneumonia* over 4-years, making difficult the assessment of the recent introduction of the pneumococcal vaccine in childhood immunization program in Rwanda. Our findings on the causal agent of CNS infection differ from that of Minjolle et al. (2002) in Iran and by others, who found *H. influenza* type B to be the most common pathogen in their studies (Mommeja-Marin et al., 2003). The reason for their findings is not clear but the finding of Shaban and Siam (2009) clearly stated that the vaccine had not been introduced at the time of their study, which explain the high isolation rate of *H. influenza* type B (Minjolle et al., 2002).

Although this is a large retrospective review over 4-years, we recognize that its retrospective nature could not allow the use of more pathogen targeted testing for identification of other rare causative agents of CNS infections. This may in part explain the high percentage (91.4%) of negative culture. Nevertheless, the study provides useful baseline data highlighting the major gap in the diagnostic capacity for CNS infection in Rwanda.

Conclusion

This cross sectional descriptive retrospective study shows low diagnostic yield of conventional microbiological methods in the diagnosis of CNS infection as 91.4% which remain undiagnosed. It provides valuable baseline data, which suggest the design of a large prospective study using both conventional microbiological and molecular methods to shed more light on the etiological agent and clinical outcome in patient with CNS infection, to help in the elaboration of better diagnostic strategies for CNS infections in Rwanda. This is a major drawback to effective algorithm for diagnosis, treatment and monitoring of drug resistance, which requires reliable diagnostic testing, highly trained staff and molecular laboratory equipment with adequate maintenance and calibration.

Novel algorithm in conventional and molecular methods could be used to detect the most common aetiologic agents of CNS like DNA/RNA virus, parasites, bacteria and fungi. The molecular method seems attractive in order to improve the detection of causative agents of meningoencephalitis for better evidence use in the treatment of patients in Rwanda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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