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LETTER

An automatic multipoint inoculator for the determination of minimum inhibitory concentrations (MICs) of antibiotics in low-income countries: a technical note

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Abstract
Multipoint inoculator is a laboratory equipment that is used to inoculate with a very high precision a certain number of microorganisms in culture media, in order to determine the minimum inhibitory concentrations (MICs) of antibiotics that would inhibit microbial growth. MICs values are crucial in the control of microbial drug-susceptibility profile for effective infectious disease control and microbial resistance stewardship. The complexity of multipoint inoculator makes it very rare or almost non-existent in developing countries laboratories. In this paper, a robust, precise and, above all, innovative automatic multipoint inoculator is developed. Otherwise, we have implemented double-sided plates with surface-mounted components. These components of Nano-electronics have the advantage that the electrical circuits do no longer have to be drilled, the components can be placed on both sides of the board, and the parasitic electrical inductances are reduced. The equipment enclosure in plexiglas facilitates its sterilization. The device carries Petri dishes that contain culture media, opens them and performs inoculation. It then closes and stores the preparations that are ready for incubation at optimal growth conditions. The present device inoculates at a given concentration of an antibiotic 21 microorganisms simultaneously.

Keywords Multipoint inoculator · Minimum inhibitory concentrations (MICs) · Surface mounted components (SMC) · Antibiotics

Introduction

The emergence of microbial infections and resistance to clinically approved antimicrobial drugs represents a substantial global threat to human health [1]. Known major factors that contribute to the emergence of these infections are socio-economically, environmentally and ecologically related [2]. In clinical laboratories microbial susceptibility tests generally rely on qualitative data based on growth inhibition around an impregnated paper disk (Kirby Bauer) in routine processes. Quantitative essays that would allow more robust therapeutic success likelihood are, however, available in certain settings (generally in research laboratories of developed countries) and usually help to access periodical changes that develop in microbial susceptibility, which is of a great relevance in addressing more accurate empirical therapy issues. In instances of resource limitation where technical and financial components are serious brakes to these advances, microbial therapy is usually based on reference data recorded from the developed world without any holistic evidences. Many authors believe that this empirical practice is also responsible for the ever-growing resistance rates in these areas, because it further exacerbates infectious diseases and related threats within the vicious cycle of poverty.

Quantitative essays like minimal inhibitory concentrations (MIC) assessment would be a key component in the

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arrays of efforts that aim at bringing microbial resistance under control in stewardship initiatives. MICs can be defined as the lowest concentration of an antimicrobial agents that inhibits the visible growth of a microorganism subsequent to incubation at optimal conditions for growth (nutrients, temperature and time, for instance). MICs are also used as a research tool to determine the in vitro activity of new antimicrobial agent [3] or to detect and confirm unusual resistance phenotypes expressed by microorganisms that are responsible for infections. Traditionally, the diffusion or dilution methods are used to perform MIC assays. In diffusion methods, a hydrophilic strip or disc is infused with antibiotic and placed in contact with the surface of an agar plate on which a microbe is grown. The antibiotic diffuses radially through the agar and displays a concentration gradient through growth inhibition close to the strip or disk. From the visual 'zone of inhibition' in this assay the MIC value can be estimated [4]. These methods are easy to perform but are not standardized to the specific characteristics of the agar growth media which otherwise may strongly influence the antibiotics diffusion. Results thereof are also subjective and variable. In dilution methods which provide a more quantitative readout than diffusion methods, microbes are inoculated in a series of separate tubes or onto separate wells (if plates are used) containing culture broth with a two-fold serial dilution of an antimicrobial agent. Unfortunately, they are generally more labor intensive and time demanding, then error prone. The use of a liquid handling robot to prepare dilution series in multiple-welled plates can reduce the time and labor involved. In this case, however, the equipment used are expensive and not widely available in labs [4]. In addition, like in microdilution, contaminants are difficult to detect. In the literature, hybrid methods combining the simplicity of diffusion methods and the quantitative results of dilution methods have emerged [4, 5]. In general, these conventional techniques assess bacteria susceptibility to antibiotics according to changes in occupied area. Thus, a positive change in areas shows that the bacteria is growing, then reflecting at first glance resistant to the drug used. This is not always true since bacteria can grow with different shapes such as filaments or swell. It is known that these changes might increase the area, while the bug is still susceptible to the antibiotic. Therefore, Choi et al. devised an imaging-based antibiotic susceptibility test performed with a single bacterial cell and the results accuracy thereof compared with gold standard broth microdilution test for several bacterial strains and antibacterial agents [6]. Another antibiotic susceptibility test that is fast enough to be used at the point of care has been developed. The authors show that the total time for antibiotic susceptibility testing, from the sample loading to diagnostic readout, is less than 30 min [7]. Other recent methods based on deep learning algorithm determining if an antibiotic inhibits a bacterial cell by learning multiple phenotypic features of the cell have been formulated [8]. Nevertheless, it has been reported some disadvantages of these methods [9]. Recently, a rapid phenotypic antimicrobial susceptibility testing (AST) in which photonic 2D silicon microarrays are used as both the optical transducer element and as a preferable solid–liquid interface for bacterial colonization has been implemented [10]. One of the equipment used to perform MICs is the multipoint inoculator. Several models of multipoint inoculators exist in the market such as the InoqulA produced by BD Kiestra, PreLUD by i2a, Innova by BD, Previ Isola by BioMérieux and WASP by Copan. However, their complexities make these devices very rare or almost non-existent in developing countries research laboratories and health facilities. In this paper, a robust, precise and above all, innovative automatic multipoint inoculator is developed. We have implemented double-sided plates with surface-mounted components (SMC). These components of Nano-electronics have the advantage that the electrical circuits no longer have to be drilled, the components can be placed on both sides of the board, and the parasitic electrical inductances are reduced. The equipment enclosure in plexiglas facilitates its sterilization. The device carries culture media in Petri dishes, opens them and inoculates 21 test microorganisms simultaneously. It closes and stores them ready to be incubated at desires growth conditions. In this essay, the antibiotic is diluted (at defined concentration) in the agar used for optimal microbial growth.

This paper is structured as follows. "Introduction" section presents the introduction. "Material and methods" section develops the design of both mechanical and electronic parts of the present inoculator. "Experimentation" section shows the prototype. "Results and discussions" section concludes and gives some perspectives.

Material and methods

Design of the mechanical part of the inoculator

The inoculator has been designed in three major steps using the SolidWorks software [11].

Module of culture medium detection

This module consists of a set of parts among which: the medium detector, the tray, the slip-ring system, the support of the slip-ring system (see Fig. 1).

– Description of the different parts of the culture medium detection module

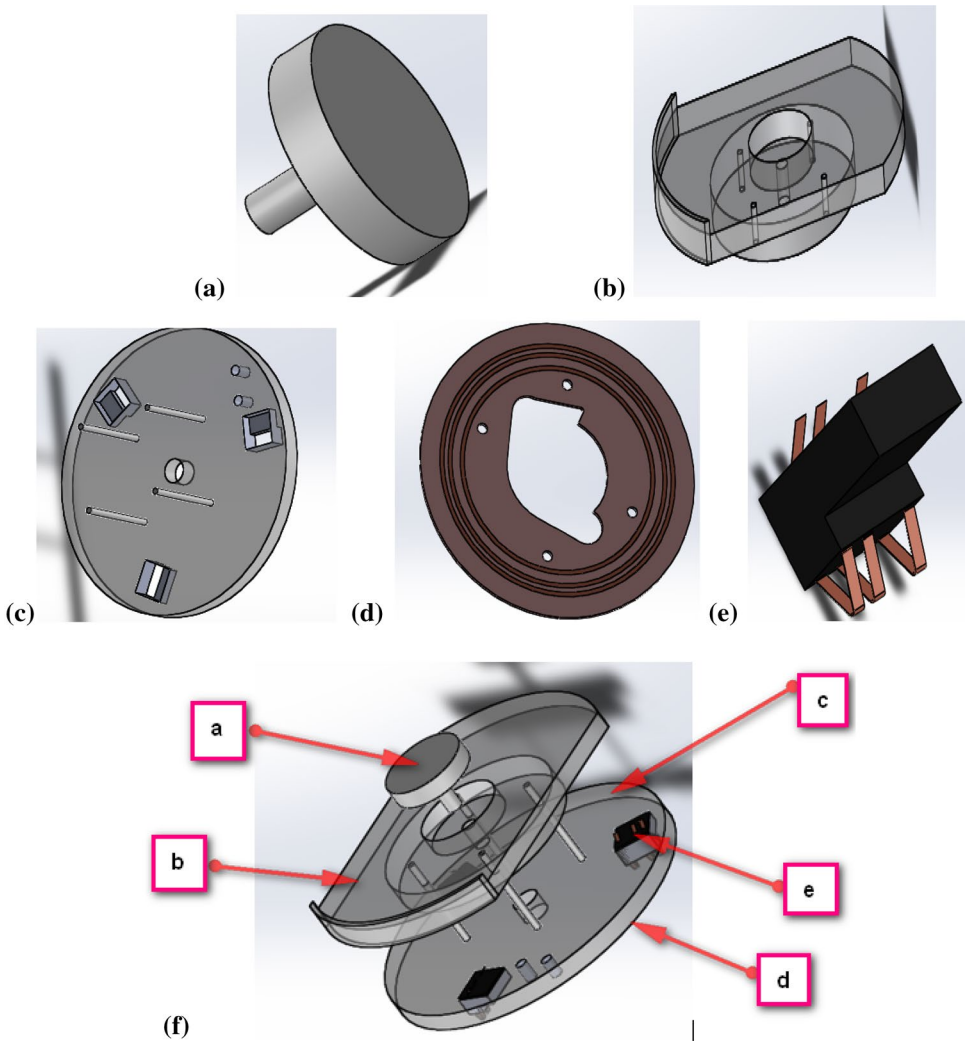

Fig. 1 Culture medium detector module: **a** Culture medium detector piece **b** tray holder **c** slip-ring system support **d** slip-ring system electronic board **e** contact part **f** assembled culture media detection module

(a) The detector of culture medium, coupled to a potentiometer. It can detect the presence of a Petri dish at the level of the tray-boxes;
(b) The tray holder, it plays the role of support during the displacement of culture medium;
(c) The support of the slip-ring system, which is set up to continually detect the presence of a Petri dish during its rotation;
(d) The electronic board of the slip-ring system;
(e) The contact part which is electrically connected to a potentiometer in order to detect at each moment during the rotation, the presence of the culture medium.

Module for the displacement of culture medium

This module just like the previous one, was modeled part by part, then assembled. It allows the movement, removal and storage of culture media. It consists of: a unipolar stepper motor, a displacement support and two steel rods and a displacement base (see Fig. 2).

– Description of the different parts of the module of displacement of culture medium

(1) The motor and screw junction holder to ensure displacement;
(2) The screw that transforms the rotational movement of the motor into translational motion;
(3) The unipolar stepper motor operating on 12 V for motion generation;
(4) Threaded rods on which the culture medium slides;
(5) Movement support when the motor is moving;
(6) The support of the module which supports to all the components of the module.

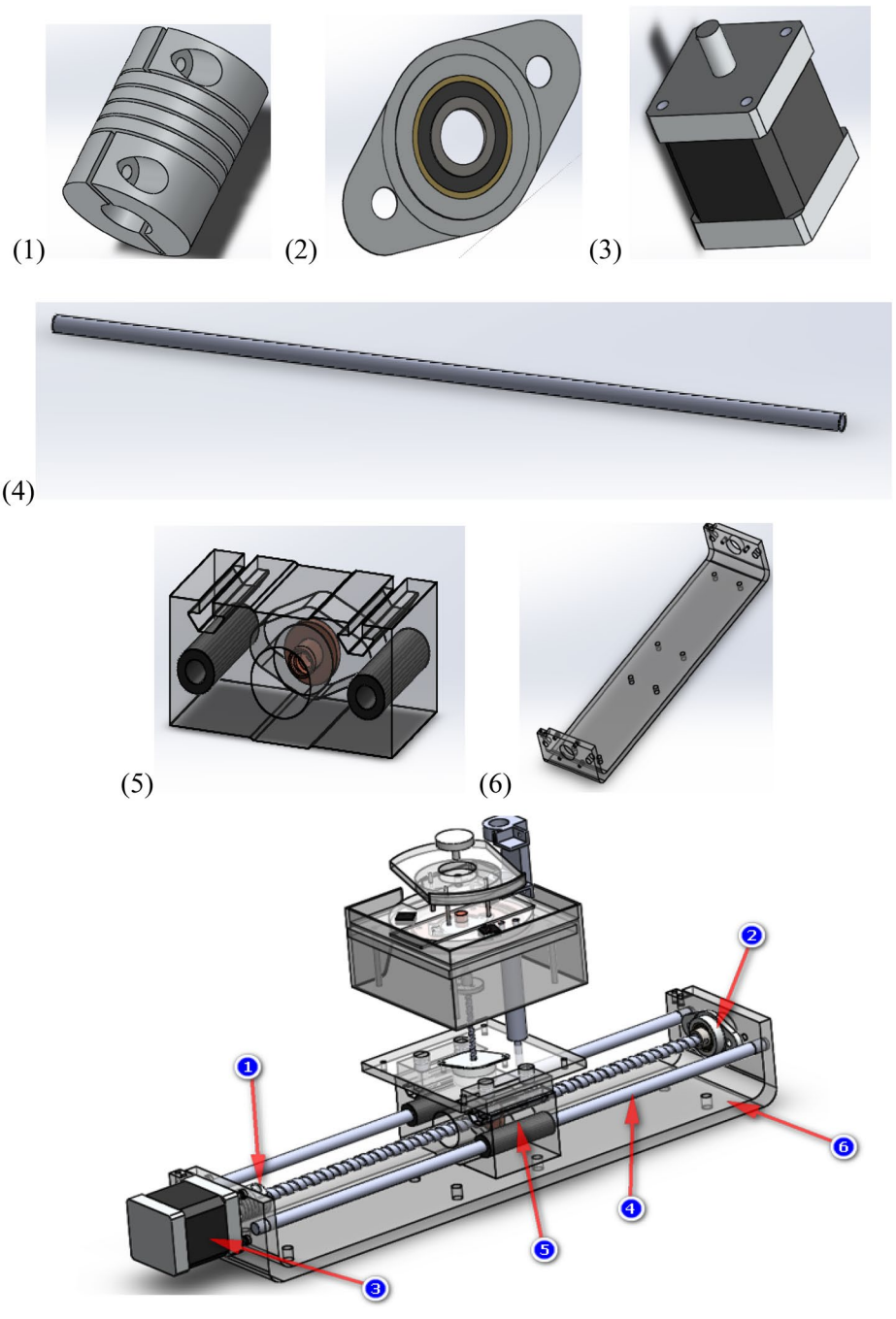

Fig. 2 Part used to adapt the motor to the base of displacement: (1) motor and screw junction holder (2) screws (3) 12 V unipolar stepper motor (4) threaded rod (5) movement support (6) displacement support (7) assembled module for the displacement of culture media

The inoculator enclosure has also been designed as it can be seen in Fig. 3a. Figure 3b presents the final equipment design in SolidWorks.

Other parts

These are power supply, infrared sensors, electrical plugs, solenoid valves and bipolar motors.

(a) Description of the other parts

– The power supply takes an input of 220 V AC main supply and output DC voltages of 12 V at 3A and 5 V at 5A. This power supply will provide all the voltage and current required for the operation of the device;

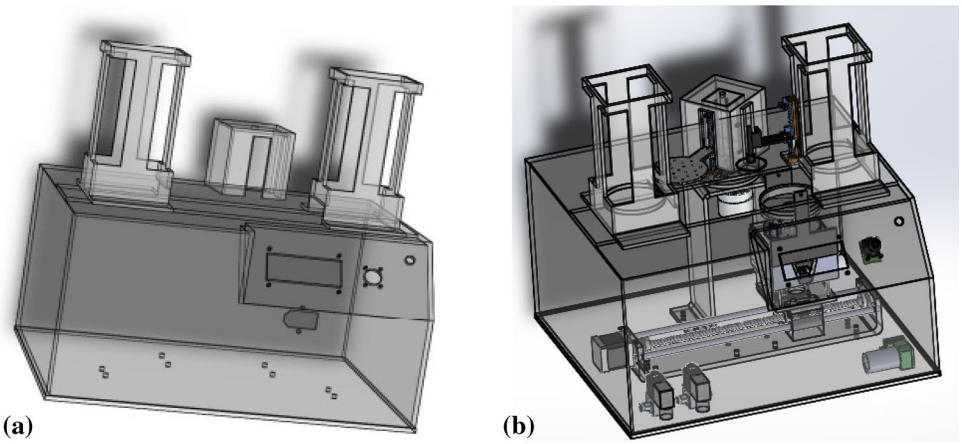

Fig. 3 The modeled system: **a** Inoculator enclosure, and **b** assembled inoculator

- The electrical plug plays the role of interface between the equipment and the arrival of the 220 V sector. It also acts as a surge protector because it consists of two fuses rated at 10A;
- The infrared sensor acts as an end of line switch. It is triggered when the moving parts reach a certain position. It is used to accurately control the movement and the position of the culture medium. It operates at a voltage of 5 V. It uses a photocell that emits a light beam. If this beam is interrupted, the sensor sends the binary value 0 to the microcontroller otherwise the binary value 1;
- The solenoid valve acts as a controlled valve for the air circuit (opening and closing circuit). Air is used for the opening and closing of the petri dish before and after inoculation;
- The elevation motor allows the elevation of the cover of the petri dish after it has been opened. This is bipolar stepper motor (4 wires) running on 5 V at 500 mA.

Design of the electronic part of the inoculator

The equipment consists of a main PCB (Printed circuit board) and five secondary PCBs, for a total of six double sided PCBs. Figure 4a shows the structure of the proposed system.

Seeding module

It is this module that allows the opening, seeding and closing of the culture medium. It consists of two modules or TB66FNPG drivers to control the two bipolar motors used for opening and seeding, terminal blocks on which will be connected the various motors, the power supply and the connection for the main board (see Fig. 4b). In this Figure, (1) represents the terminal blocks and (2) represents the two TB66FNPG drivers.

Positioning module

This is the module responsible for carrying the petri dish containing the culture medium, to position it for seeding and finally to storage. It consists of two unipolar stepper motors, a servomotor and a slip-ring system. Its board consists of a set of resistors, an ULN2003A for the sequential control of the stepper motor, LEDs to be able to visualize the sequence of the motor and terminal blocks for the interconnection of the various components of the board and the connection to the main board. The peculiarity of all these boards is that they are double-sided, having components mounted on the surface. It can be observed in Fig. 4c, surrounded in red, miniaturized electric resistors and in blue a miniaturized ULN2003A. These are surface mounted components (SMD).

Seeding support module

This module is made to control our rotation motor as well as its various positioning sensors. It is this motor that will rot the seeding arm to collect and seed the bacteria. It consists of a set of four IRFZs that are MOSFETs.

Main electronic board

The main board consists of a microcontroller, the different terminal blocks to which are connected the control pins for each secondary board (see Fig. 4d). Its schematic is shown in Fig. 4a.

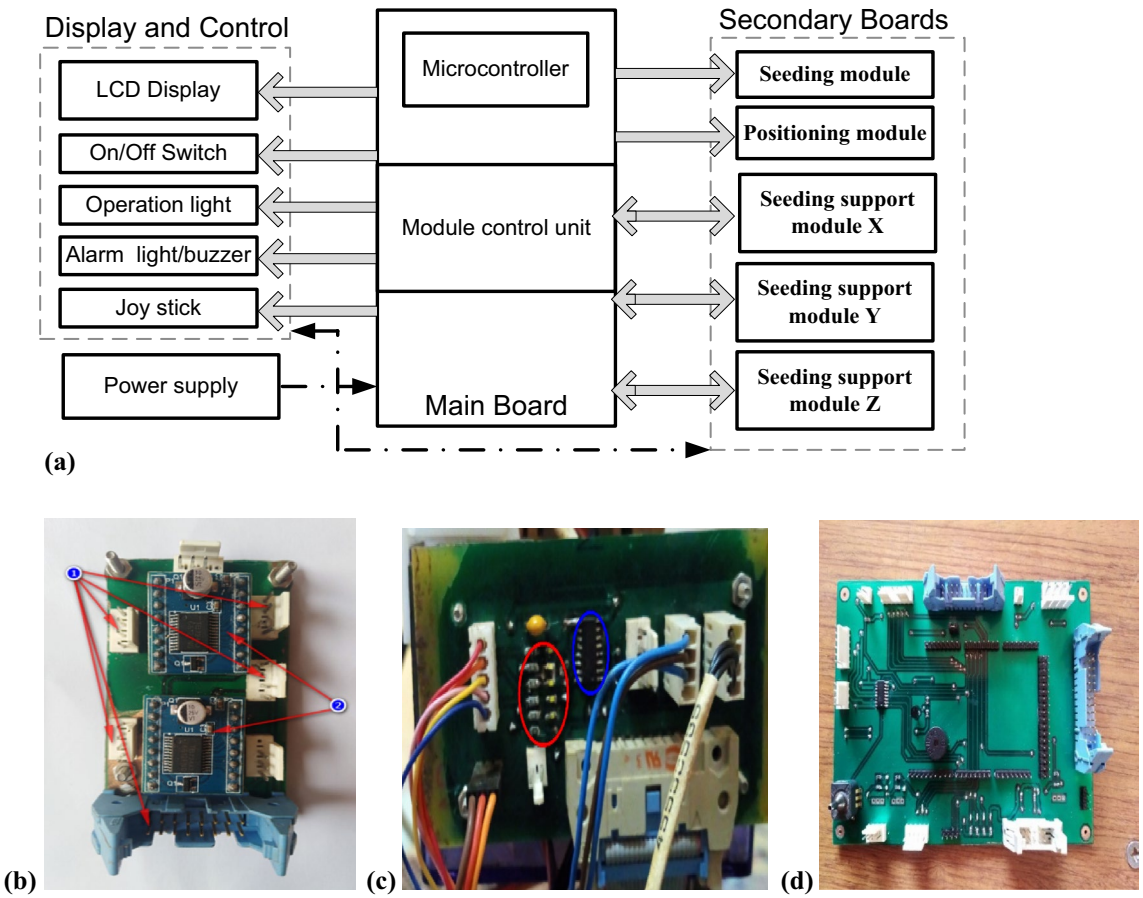

Fig. 4 The system representation. **a** the structure of the system **b** Seeding module PCB **c** positioning module PCB **d** main controller PCB

Experimentation

A serial dilution was conducted in order to have Ampicillin concentrations in Muller Hinton agar ranging from 32 µg/mL through 0.125 µg/mL in Petri dishes. Bacterial suspension (at 0.5 McFarland) from 21 bacterial strains were then prepared and inoculated by the device on the agar containing the antibiotic at various concentrations. In total, 11 Gram negative rods, 8 Gram positive cocci and two reference bacterial strains (*E. coli*: ATCC 25,922 and *S. aureus*: QC 1625) were used. The preparations were then incubated for 24 h at 37° C. Upon incubation completion the results appeared as shown in Fig. 5c. Results were interpreted as conducted in reference [12].

Results and discussions

The developed inoculator has been assembled and the different views of the final equipment can be observed in Fig. 5a, b. It is currently in use in the research laboratory of the 'Cliniques Universitaires des Montagnes' in Bangangté, in the West region of Cameroon. In this work, 21 microorganisms have been tested at the same concentration of an antimicrobial drug simultaneously with the equipment. The results can be found in Fig. 5c.

It can be observed in Fig. 5c that by increasing the concentration of an antimicrobial drug progressively, it is possible to determine the MIC for each microorganism. The red arrow of Fig. 5c displays images of growth inhibition that occurs progressively with increasing antibiotic concentration rightward. With regards to other methods which are otherwise more demanding and error prone (microdilution or macrodilulion, for instance) commonly used to assess MICs in instances of resource limitation, the use of present tool will facilitate MIC assessment in middle sized research laboratories of developing countries and help implement microbial resistance stewardship programs. The performance of a multipoint inoculation system is measured through clinical microbiological tests such as yield factor, degree of isolation of colonies, reproducibility and cross contamination [13]. In addition, the cost of the equipment is also a huge determinant factor of comparison. Specifically in this work,

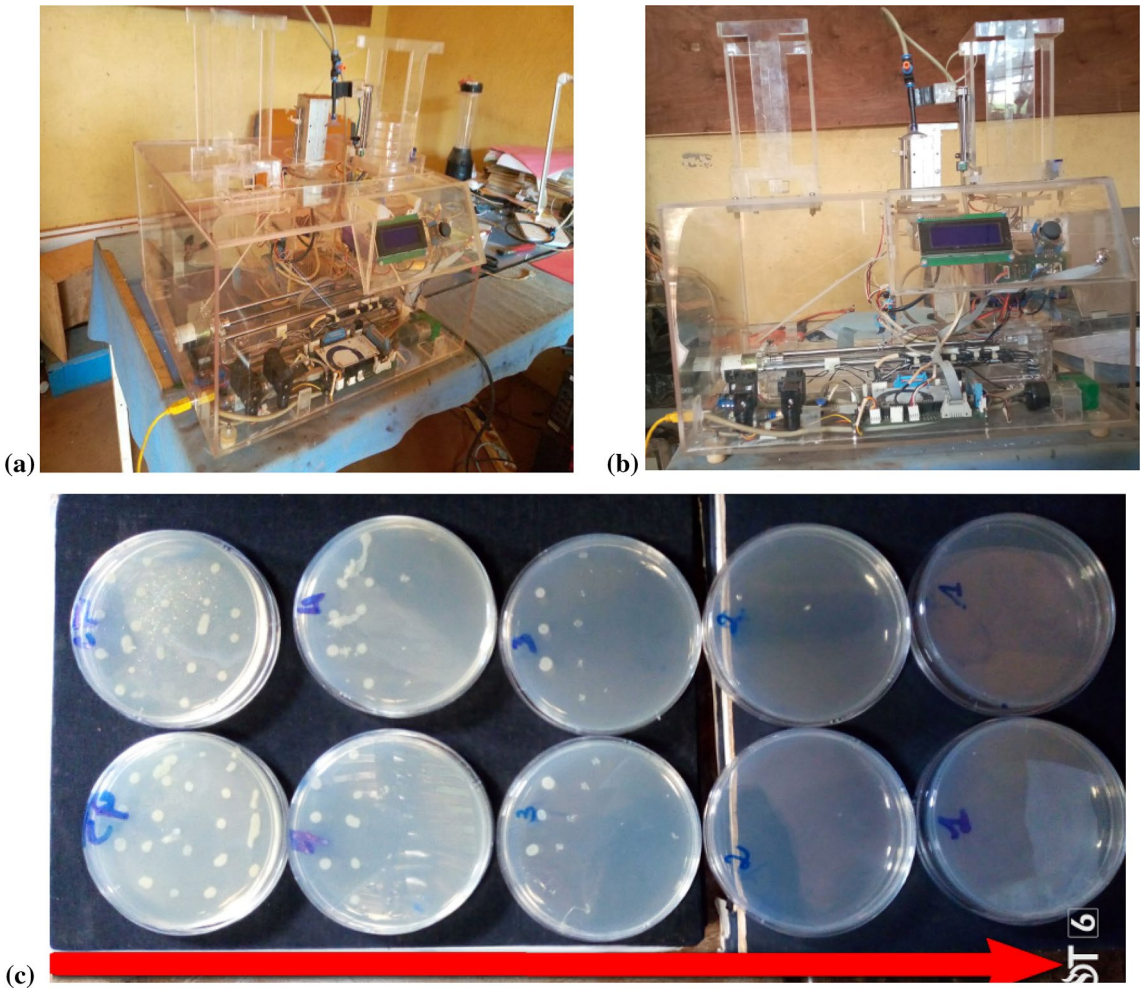

Fig. 5 Different views of the developed inoculator (**a**, **b**) and MIC test performed with the inoculator (**c**) (at increasing antibacterial concentration)

much importance is given to the cost factor while the other performance criteria are left for future investigation where the proposed system will be compared to existing systems.

Conclusion

An automatic multipoint inoculator has been developed in this work in order to determine the minimum inhibitory concentrations (MICs) of antibiotics. The technology of double-sided plates with surface-mounted components (SMC) has been successfully implemented. The equipment has been used to test 21 microorganisms simultaneously at given antimicrobial drug dilutions and promising results have been recorded. This device could improve microbial susceptibility tests in clinical laboratories in low-income countries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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