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A thermal ink-jet printing approach for evaluating susceptibility of bacteria to antibiotics

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ABSTRACT

An inexpensive method for determining minimum inhibitory concentrations (MIC) using ink-jet printing to deposit drug solutions and bacterial suspensions onto agar was developed. Substrate concentrations were varied using a "Y-value", whereby a series of rectangles with the same width and colour but different heights were printed within a fixed unit area. Prior to MIC determination, the printer cartridges used were calibrated using Fast Green dye. The impact of thermal ink-jet printing on bacterial viability was assessed by colony counting and found not to be deleterious. MIC determinations were conducted by printing varying concentrations of the antibiotics onto agar-coated glass slides then printing a thin even film of a known bacterial density of *Lactobacillus acidophilus*. Broth microdilution was performed simultaneously to validate the results. Slides and well plates were then incubated anaerobically for 48 h. The MIC values obtained for the antibiotics used were within a permissible range for comparison.

1. Introduction

Antibiotic resistance is a major public health issue worldwide. Misuse of antibiotics on the part of patients, i.e., failure to complete therapy, re-use of leftover antibiotics or skipping doses, can result in suboptimal concentrations in blood and the development of antibiotic resistance (Kardas et al., 2005). Similarly, the wrong indication of antibiotics on the part of prescribers can also result in antibiotic resistance (Richman et al., 2001). The issue of wrong indications can be addressed by assessing the susceptibility of micro-organisms to antibiotics to gauge the clinical effect as well as effective dose of antibiotics before administration (Lamy et al., 2004). Minimum inhibitory concentration (MIC) determinations, defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of micro-organisms after overnight incubation, can be conducted to ascertain susceptibility (Andrews, 2001). Data from MIC determinations as well as the pharmacokinetics and pharmacodynamics of antibiotics can help prescribers to know which agent will be effective in combatting infection in addition to the dosage determination (Wexler, 1991; Turnidge and Paterson, 2007).

MIC determination has been described as the 'gold standard' for determining the susceptibility of organisms to antimicrobials and is, therefore, used to judge the performance of all other methods of susceptibility testing (Lamy et al., 2004). MIC determinations are also performed when patients have severe infections, e.g. meningitis, and during unexpected treatment failure. When new antibiotics are discovered, MIC determinations are usually conducted to assess efficacy (Acar and Goldstein, 1996). Manual methods like agar dilution, broth dilution, and broth microdilution can be used in MIC determination (Andrews, 2001). The difficulty and workload involved in obtaining varying drug concentrations on a large scale as well as the possibility of errors in preparation of antibiotic solutions are major drawbacks with these techniques (Wexler et al., 1991; Jorgensen and Ferraro, 2009). Automated approaches like the spiral gradient endpoint (SGE) technique and more recently the HP D300 digital dispenser are available for determination of MIC. These automated approaches are, however, very expensive hence, a major challenge in resource-restricted settings (Wexler, 1991; Smith and Kirby, 2016).

Since any apparatus for MIC determination should be able to deposit solutions with high accuracy and to create concentration gradients we wondered whether thermal ink-jet printing (TIJP) may be a useful technology. TIJP is a non-contact technique originally developed for production of digital data images on a substrate (Ferris et al., 2013). TIJP offers advantages like high throughput and high reproducibility (Wilson Jr and Boland, 2003; Lemmo et al., 1998; Alper, 2004). It is cheap and thermal ink-jet printers can work with very low volumes of

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solution (20 μL), which is useful in the pharmaceutical industry when the amount of materials being investigated are frequently limited (Allain et al., 2004; Derby, 2008). In the context of MIC determination, because TIJP allows controlled deposition of aqueous media, it should allow precise dosing of bacterial suspensions and/or active pharmaceuticals onto growth media, including agar plates and it also has the potential to print concentration gradients. Thus, the specific aim of this work was to evaluate the potential of TIJP for MIC determination.

2. Materials and methods

Lactobacillus acidophilus (LA - 5) was obtained as a pure culture from the Christian Hansen Culture Collection (Reading, UK). The antibiotics used, pure amoxicillin, tetracycline, doxycycline, and ampicillin (92.5–100.5% purity), were from Sigma-Aldrich, UK.

2.1. Printer modification

The thermal ink-jet printer used in this work was the Hewlett-Packard (HP) 5940 Deskjet. This printer was chosen on the grounds of (i) cost: the model is relatively inexpensive hence fits into the motive of developing a cost-effective technique; (ii) generation of model: being an older HP model, the printer design is simple and offers a greater ease of modification; and (iii) cartridge properties: the HP 5940 model offers a separate compartment for both black and colour cartridges and either cartridge can work without the other present.

The printer was modified such that rather than the substrate (paper in the unmodified printer) passing through the printer's rollers during operation, printing is done onto a stage mounted underneath the cartridge print head without the printer detecting the absence of paper. The key point with the printer modification is to identify the printer's sensors and manually activate these when needed. The cartridges used were HP black ink cartridges number 337. Black cartridges were used and not tricolour cartridges because tricolour cartridges produce droplets of different sizes from the different compartments. These droplet sizes are not user controllable, however, with black cartridges, droplets are produced from one chamber and are of the same size. This presents a better option in assay development (Buanz et al., 2013).

The modifications to the printer mean that when printing an image, the substrate does not move vertically. In other words, all the deposited solution by the printer is in a single band with a maximum width equal to the size of the nozzle plate on the print cartridge. Changing the volume of solution being deposited is achieved simply by changing the dimensions of the rectangular template used to initiate printing. The width of the rectangle is kept fixed and the height varied for the series of rectangles. Since the height is conventionally the y-axis, we denote this term as the 'Y-value'; with each Y-value corresponding to the height of the rectangle in cm. The term 'Y-value' was used to define how much solution was printed because the height is the only parameter varied for the series of rectangle templates used to drive the printer. An illustration of the Y-value concept is shown in Fig. 1, where three rectangles have the same shade of black (100%) and width (0.5 cm) but the height (Y-value) changed from 0.5 cm to 1.5 cm. Printing these templates onto a fixed area results in a linear increment in the volume of solution deposited (Vuddanda et al., 2018; Alomari et al., 2018).

2.2. Print substrate

Agar-coated glass slides were chosen as the print substrate (biopaper) to provide nutrients for cell growth. Microscope glass slides (Thermo Scientific, UK), frosted at one end, were used to prepare print substrates. This type was chosen to make room for holding the slide (the frosted end) without touching the agar. Sterilised de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) (1 mL, 50 °C) was pipetted onto one end of the sterilised glass slide and held at an acute angle to allow the agar to flow gently towards the other end excluding the frosted area. This created an even thin film of agar on the slide. The agar was allowed to set and the procedure was repeated such that each slide had 2 mL (two 1 mL) MRS agar films on it. The agar-coated glass slides were then stored in sterile petri dishes.

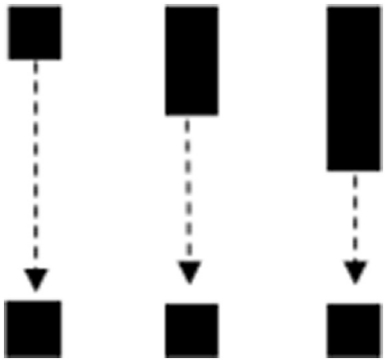

Fig. 1. Illustration of the Y-value concept whereby three rectangles having the same colour and width but varying height from 0.5 cm to 1.5 cm are printed onto a fixed unit area.

2.3. Disinfection technique

70% ethanol was used to disinfect the cartridges prior to use. The inner chamber and outer parts of the cartridges were rinsed repeatedly with sterile deionised water then sprayed with 70% ethanol with greater attention given to the nozzles. Cartridges were dried completely afterwards under aseptic conditions. There were no colonies after the incubation of imprinted sterile deionised water onto Iso-Sensitest agar (Oxoid, UK) highlighting efficacy of the disinfection technique.

2.4. Effect of thermal ink-jet printing on bacterial cell population

L. acidophilus (10^7 Colony Forming Units (CFU)/mL, 0.5 mL) was added to phosphate-buffered saline (PBS, 4.5 mL) (Fisher, UK) to create a 10^6 CFU/mL bacterial culture. The suspension (1 mL) was then put into the ink chamber of a sterilised cartridge and used for printing. The printing template used was a 2 cm × 20 cm black rectangle. Printing was done into a sterile petri–dish mounted on a stage 2 cm from the print head. The printing process was repeated several times until the volume of liquid in the petri-dish was enough to enable withdrawal of 50 μL. This was then serially diluted (1 in 10) six times. The resulting solution was then plated out onto MRS agar and incubated for 48 h. Two cartridges were evaluated and the experiment repeated three times. The initial number of bacteria in the sample was also determined simultaneously for each experiment.

2.5. Standardisation of cartridges

The objective of standardising cartridges was to know the volume of solution the cartridges deposited for a given Y-value. Fast Green dye solution (1 mg/mL) was prepared and used as a standard. Y-values of 0.5, 1, 2 and 3 cm with a constant width of 0.2 cm were printed individually onto clean acetate sheets. The acetate sheets were carefully cut into bijous and deionised water (1 mL) was added to dissolve the dye. The bijous were vortexed to ensure dissolution of the dye. The solutions were then transferred into vials for analysis with high-performance liquid chromatography (HPLC).

A gradient system was adopted with acetonitrile (HPLC grade) as the organic phase and 55 mM acetate buffer (pH 5 ± 0.02) as the aqueous phase at a flow rate of 1 mL/min for 10 min. The gradient system consisted of 15% acetonitrile and 85% buffer for 6 min then 60% acetonitrile and 40% buffer for a minute after which 15% acetonitrile and 85% buffer was run again for 3 min. An injection volume of

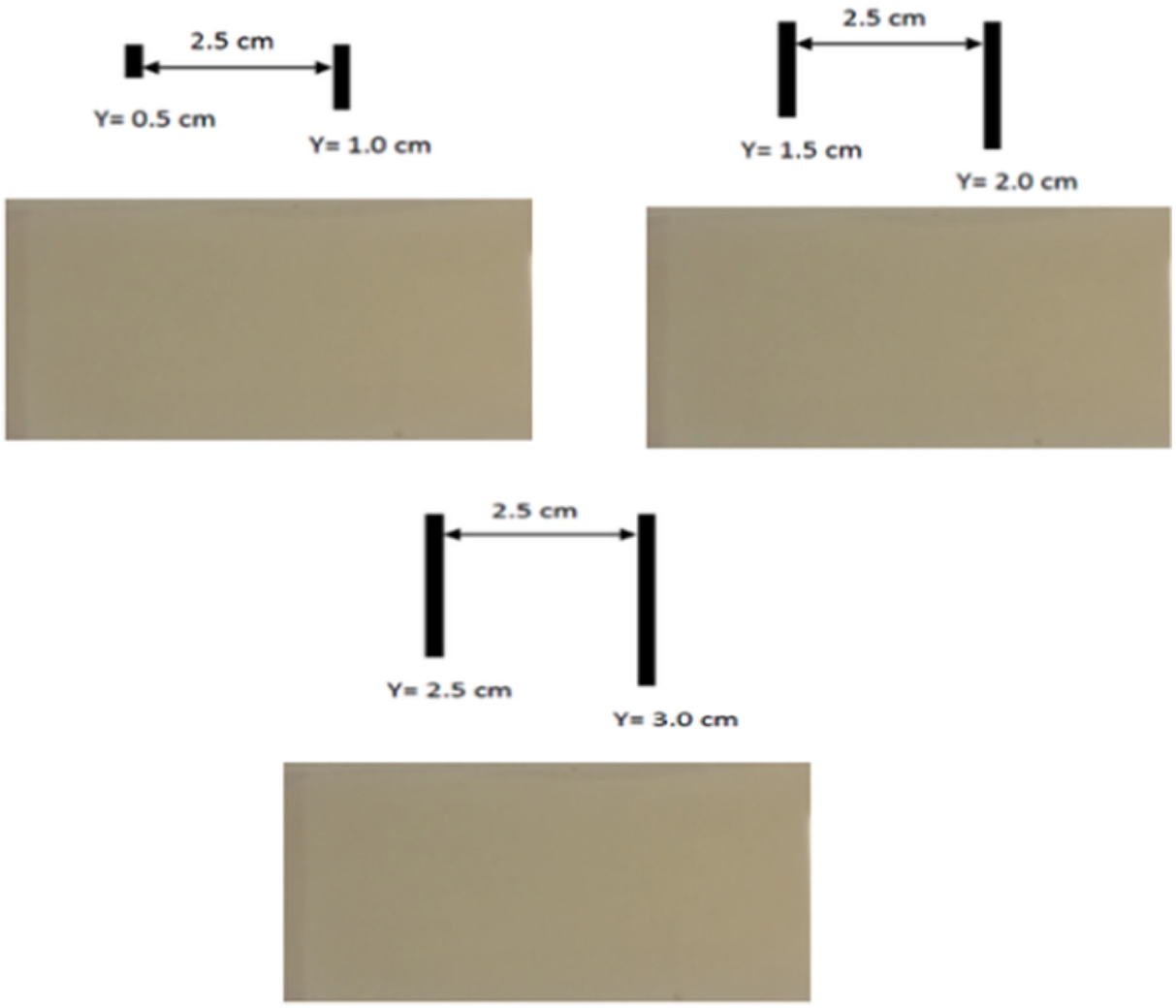

Fig. 2. Illustration of designed minimum inhibitory concentration determination model. Each Y-value set was printed onto an agar-coated glass slide with an interval of 2.5 cm between each set.

10 µL was used with the column temperature set at 30 °C. A wavelength of 600 nm was used for detection.

A calibration curve for Fast Green dye in the range 2 to 0.0625 µg/mL was prepared with the same HPLC parameters described above, with an excellent linearity (r^2 = 0.9999) obtained.

2.6. Determination of MIC

An aliquot (1 mL) of a solution of known antibiotic concentration was put into the ink compartment of a sterilised black cartridge. This was used to print the Y-value set (0.5 cm and 1 cm), (1.5 cm and 2 cm) and (2.5 cm and 3 cm) onto MRS agar-coated slides on a stage 2 mm from the cartridge head (Fig. 2). The different Y-values had an interval of 2.5 cm between them to account for any possible drug diffusion; each set was duplicated. The cartridge was then removed and the main parts of the printer that came into contact with the cartridge were sprayed with 70% ethanol and allowed to dry. *L. acidophilus* (10^5 CFU/mL, 1 mL) was put into another sterile cartridge and used to print an even film (1 cm × 5 cm rectangle) of bacteria across all the slides. A control slide was also set up which had bacterial film with no drug. The experiment was repeated with varying antibiotic concentrations. Each concentration was prepared in triplicate, hence there were six determinations per Y-value. The slides were then incubated under anaerobic conditions for 48 h. Broth microdilution was simultaneously performed using a 96-well plate and the results used to validate TIJP results. The antibiotics used were ampicillin, tetracycline, amoxicillin and doxycycline.

2.7. Effect of inoculum density on MIC

The protocol as detailed above (determination of MIC) was followed with one set having an inoculum density of 10^5 CFU/mL and another set having an inoculum density of 10^6 CFU/mL. The experiment was carried out using ampicillin and tetracycline against *L. acidophilus*.

2.8. Effect of varying contact time between bacteria and drug on MIC

In the model designed, the bacteria and drug were placed into separate cartridges, so investigations were carried out to determine whether the time involved in changing cartridges and disinfecting key parts of the printer influenced the MIC. Three different sets of experiments were conducted using the protocol above. In the first set, tetracycline was printed and a period of 20 min allowed before the bacteria were printed. 20 min was chosen because it was sufficient to cater for cartridge change and disinfection. In the second set the bacteria were printed first then tetracycline immediately afterwards. In this instance, the jetted antibiotics come in contact with bacteria right after printing. A third set was conducted whereby an interval of 48 h (duration of incubation) was allowed between drug deposition and bacterial printing.

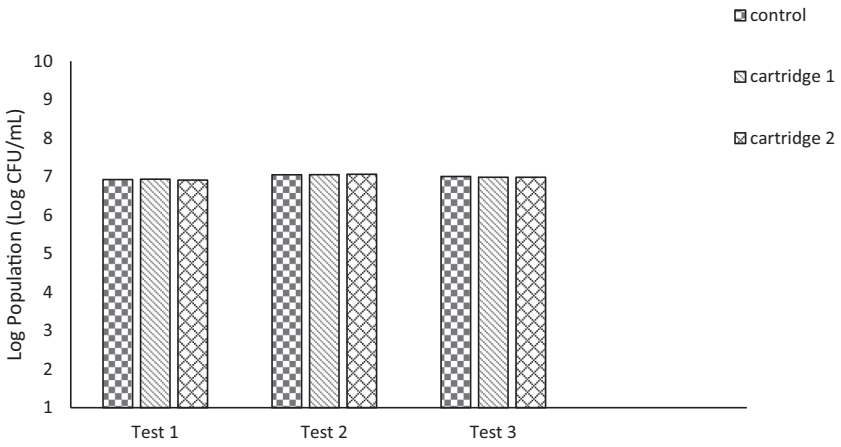

Fig. 3. Effect of thermal inkjet printing *Lactobacillus acidophilus* on bacterial viability (y-axis, cell number expressed as log colony forming unit (CFU)/mL; x-axis, triplicate sampling of two independent cartridges; control represents the original cell number before printing).

3. Results

3.1. Effect of thermal ink-jet printing on bacterial cell population

The effect of thermal ink-jet printing *Lactobacillus acidophilus* on the bacterial viability yielded similar results between the two cartridges and the control (Fig. 3). Log counts of 6.92 ± 0.01, 7.05 ± 0.01, and 6.99 ± 0.01 colony forming units/mL were obtained for the triplicate determinations.

3.2. Standardisation of cartridges

The calibration curve for Fast Green dye is shown in Fig. 4A with a linear plot obtained between 0.0625 and 2 μg/mL. The corresponding plot of Y-value against area under curve (AUC) was also linear (r^2 = 0.9992), validating the Y-value concept (Fig. 4B). The volume of liquid deposited by ink-jet printheads usually contains a solute that is a fraction of the total solute in cartridge. This fraction is fixed per template and it is denoted here as the characteristic ratio per template. This was obtained by expressing the amount of Fast Green dye per Y-value as a fraction of the cartridge concentration used in printing Y-values (Eq. (1)). A plot of the characteristic ratio against the Y-value for the templates used is shown in Fig. 4C.

$$\text{Characteristic ratio} = \frac{\text{equivalent concentration}}{\text{cartridge concentration}} \quad (1)$$

3.3. Determination of MIC

Fig. 5 shows images from MIC determination for ampicillin using a concentration of 0.2 mg/mL in the cartridge. Fig. 5A shows an even strip of bacterial colony after incubation for the control (no antibiotic was deposited). Fig. 5B shows a similar colony pattern to the control even though there were antibiotics printed indicating the concentration of antibiotic present was not enough to inhibit growth of *L. acidophilus*. Two distinct zones are seen in Fig. 5C, representative of growth inhibition in the areas where antibiotic was printed. Fig. 5D showed almost no growth on the slide. Table 1 shows the results of microbial growth or absence of growth for various concentrations of antibiotic solutions against *L. acidophilus* after incubation.

Table 2 shows the calculated and corrected (obtained by using next incremental doubling dilution for the calculated) MIC values from the test concentrations in comparison with values obtained via broth microdilution.

A sample calculation for the calculated MIC value using 0.6 mg/mL tetracycline is shown below (Eq. (2)).

$$\text{MIC at 2.5cm} = 0.6 \times (\text{characteristic ratio at 2.5 cm}) \quad (2)$$

From graph (Fig. 4C) characteristic ratio at 2.5 cm ≡ 0.00098

$$\text{MIC} = 0.6 \times 0.00098 = 5.9 \times 10^{-4}\ \text{mg/mL}\ (0.59\ \mu\text{g/mL})$$

3.4. Effect of inoculum density on MIC

Table 3 shows the effect of inoculum density on MIC. It was observed that when the inoculum density was increased from 10^5 CFU/mL to 10^6 CFU/mL an increment of 0.5 cm Y-value was observed for all MICs except for 0.7 mg/mL tetracycline.

3.5. Effect of varying contact time between bacteria and drug on MIC

Comparable results were obtained when tetracycline was printed immediately onto bacteria and when an interval of 20 min (to make up for time used to change cartridges and disinfect vital parts of printer) was allowed between antibiotic deposition and bacterial prints (Table 4). In both instances, a cartridge concentration of 0.3 mg/mL yielded no growth inhibition whilst 0.7 mg/mL cartridge concentration resulted in growth inhibition at a Y-value of 2.0 cm (equivalent to 0.55 μg/mL). When an interval of 48 h was allowed between printing of drug and antibiotic, no growth inhibition was observed and the nature of bacterial growth on all slides for both 0.3 mg/mL and 0.7 mg/mL cartridge concentrations were like that of the control.

4. Discussion

The primary concern when using TIJP to print bacteria is whether cell viability is maintained after transit through the print head because the heating elements reach a significant temperature (about 200 °C) during droplet formation (Allain et al., 2004). Although the temperature at the surface of the heating element is high, the actual temperature rise in the printing solution is significantly smaller, because the heat acts to create expanding gas bubbles that drive the droplet from the nozzle and the bubbles effectively insulate the solution. Xu et al. (2005) showed that viable Chinese hamster ovary cells (mammalian cells) could be printed with a viability of 90% and that the effective temperature of the solution (bio-ink), did not rise more than 10 °C above ambient conditions. Since mammalian cells can be successfully printed, bacteria should similarly be unaffected by the process of ink-jet printing. In our work, very little loss in viability was seen when evaluating the effect of thermal ink-jet printing on bacterial cell population.

Verification that the amount of solute printed varied linearly with the Y-value was performed and confirmed using a coloured standard

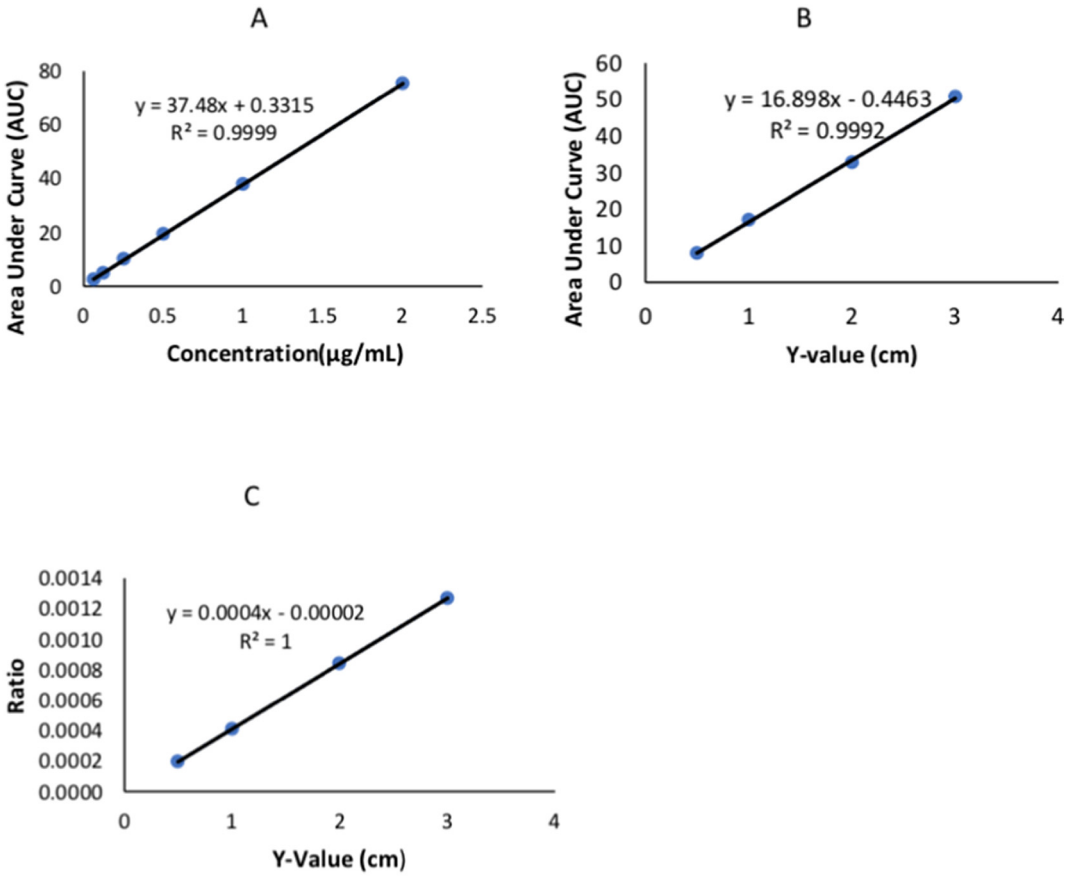

Fig. 4. A) Calibration curve of Fast Green dye between 0.0625 and 2 µg/mL. B) Plot of Y-value against area under curve (AUC). C) Plot of Y-value against characteristic ratio, the characteristic ratio was obtained by expressing the amount of Fast Green dye per Y-value as a fraction of the cartridge concentration used in printing Y-values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fast Green dye). We have previously used the Y-value concept in the personalisation of warfarin therapy and printing combination of triiodothyronine and thyroxine (Vuddanda et al., 2018; Alomari et al., 2018). The linear plot obtained for the Y-value and the characteristic ratio facilitated the direct extrapolation of the amount of drug deposited for each Y-value.

Once this approach is validated, the MIC can in principle be determined from the minimum Y-value at which growth is inhibited. An assay was developed whereby two strips were printed with different Y-values per agar-coated glass slide. *L. acidophilus* was the test organism used due to its generally recognised as safe (GRAS) status and the antibiotics used were ampicillin, tetracycline, amoxicillin and doxycycline.

The MIC, in the scenario illustrated in Fig. 5, is the concentration of drug at the Y-value of 1.5 cm since this is the least concentration with no bacterial growth.

The MIC obtained by the broth microdilution method was recorded in the standard manner; i.e., if growth was observed at 0.5 µg/mL and no growth was observed at 1 µg/mL, the MIC was documented as 1 µg/mL. However, to account for the fact that concentrations increased in linear, rather than doubling increments when printing, MICs were rounded off (corrected) to the next incremental doubling dilution for analytical comparisons with broth microdilution. A similar concept is usually adopted when comparing SGE with standard techniques (Hill and Schalkowsky, 1990). Since the TIJP model being designed was similar to the SGE technique, the same approach of comparison is adopted.

The corrected MIC values from the test concentrations used were all within one doubling dilution from the broth microdilution MIC, which were within the range of acceptable MIC values when comparing with a standard technique. (Pong et al., 2010; Wexler et al., 1996; Paton et al., 1990; Andrews, 2001; Jorgensen and Ferraro, 2009). As a model still in developmental stages, this was a positive outcome for comparable results to have been obtained within the range for controlled and defined delivery of bacteria by the ink-jet.

The inoculum density is an important factor that influences MIC (Amsterdam, 1996; Andrews, 2001). The higher the inoculum density the greater the chances of having resistant strains present in the population, hence, the probability of having a higher MIC. Bacterial populations ranging from 10^8 CFU/mL to 10^9 CFU/mL have been used in inoculation using SGE (Pong et al., 2010; Paton et al., 1990; Wexler et al., 1996). However, in this work only two bacterial concentrations were investigated (10^6 CFU/mL and 10^5 CFU/mL), because these two gave controlled delivery with little or no satellite droplets with the designed bacterial inoculation template in optimisation experiments.

When the effect of inoculum density was ascertained, the relatively higher equivalent MIC values for the 10^6 CFU/mL inoculum was expected because a slightly greater bacterial population was being acted upon by the same amount of antibiotic as used for 10^5 CFU/mL. However, when these MIC values were corrected by rounding up to the next doubling dilution for comparison with standard techniques, the values obtained for ampicillin using 10^5 CFU/mL inoculum (0.125 µg/mL) and 10^6 CFU/mL inoculum (0.25 µg/mL) varied by one doubling dilution. The MIC obtained for the 10^6 CFU/mL inoculum was, therefore, equal to that for the broth microdilution obtained in Table 2. For tetracycline, however, the same MIC was observed for both inoculum densities i.e., 1 µg/mL for both 10^5 CFU/mL and 10^6 CFU/mL inoculum densities. A plausible explanation for this is the fact that as the MIC values increase the range between successive doubling concentrations increase hence, the likelihood for slightly higher MIC values obtained

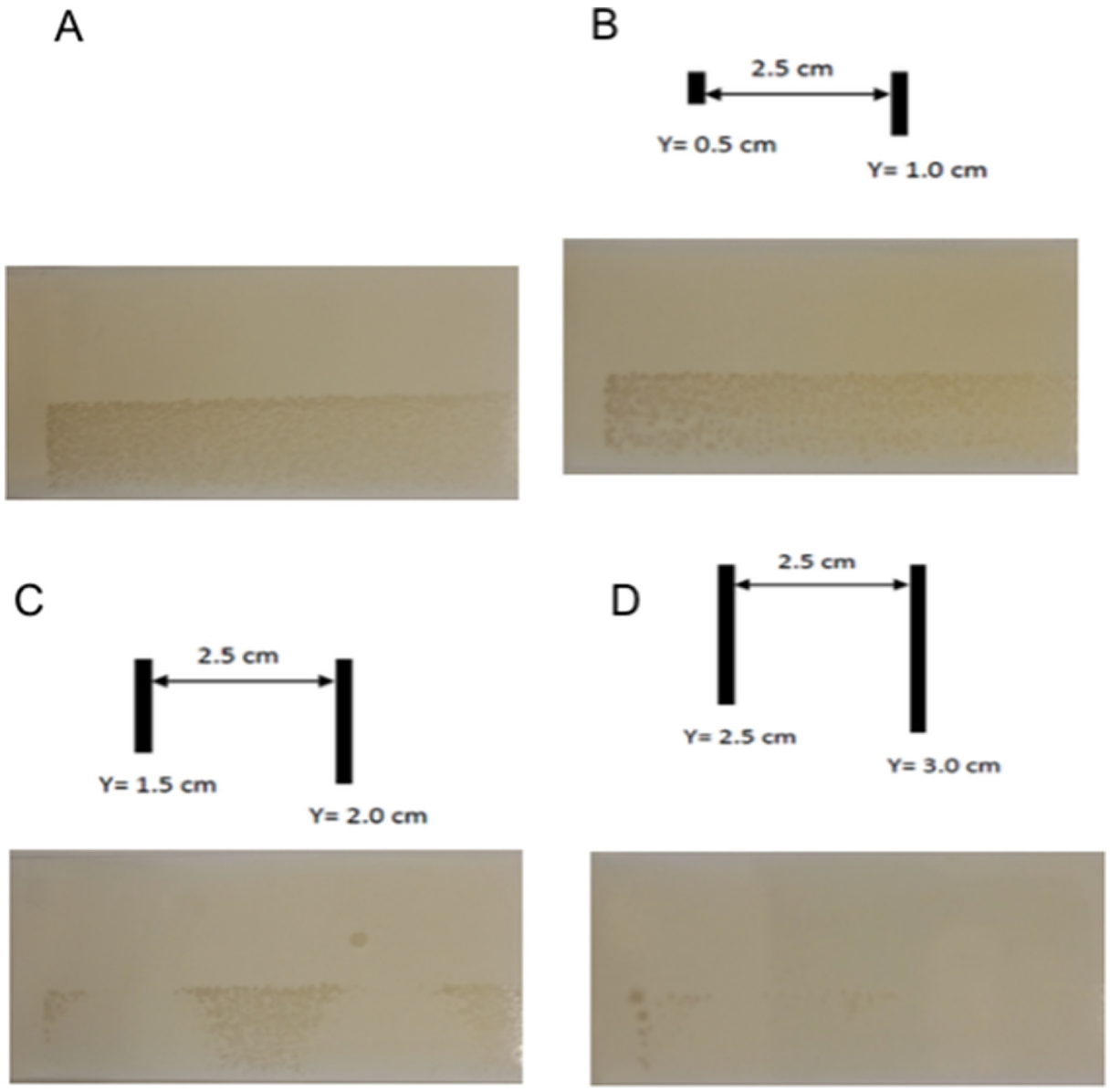

Fig. 5. Illustration of minimum inhibitory concentration on agar-coated glass slides after incubation; A) Control B) 0.5 cm and 1 cm Y-value C) 1.5 cm and 2 cm Y-value D) 2.5 cm and 3 cm Y-value. In this scenario, areas of growth inhibition were observed on images C and D.

via TIJP to be similar when corrected to the next doubling concentration irrespective of the change in inoculum density. A drawback with the use of 10^6 CFU/mL inoculum was the satellite drops on the agar-coated slides, hence 10^5 CFU/mL was used for subsequent fine tuning of the model.

Investigations conducted to find out whether the time of contact between antibiotics and bacteria played a role in MIC determinations gave interesting findings. The fact that no growth inhibition was observed for 0.3 mg/mL immediately onto bacteria and when an interval of 20 min could be because the effective concentrations on the slides were sub-inhibitory. Growth inhibition was, however, observed when 0.7 mg/mL cartridge concentration was used at a Y-value of 2.0 cm indicating that there was effective antibiotic available within the time period. The absence of growth inhibition observed for both 0.3 mg/mL and 0.7 mg/mL after bacteria were printed 48 h after drug deposition could be because the effective drug concentration on the slides decreased over the duration of the incubation highlighting the time-dependent nature of the experiment.

This time dependence also implied that unlike other susceptibility testing techniques (agar dilution) whereby media with antibiotics incorporated can be stored for future use, it will be inappropriate with this technique as the volume of antibiotic solution incorporated onto agar-coated slides is very small. Hill and Schalkowsky (1990) showed that MICs did not vary significantly when an interval of interaction up to 7 h was allowed between deposition of drug and organism. However, since relatively smaller quantities of antibiotics were used here in comparison to the SGE technique, the potential impact of time on the MIC can be very significant. Also, in instances where anaerobic species are being investigated, it is better to keep the interval of interaction to an acceptable minimum as these organisms require a longer generation time and false negative results could potentially be obtained. It is, therefore, recommended that for consistency in MIC results, it is best to perform tests within a time frame of 20 min.

5. Conclusion

A thermal ink-jet printer was modified to enable printing of bacterial suspensions and drug solutions. Despite the printer being of a

Table 1
Results for minimum inhibitory concentrations of antibiotics against *Lactobacillus acidophilus* determined via thermal ink-jet printing.

Antibiotic	Cartridge concentration (mg/mL)	Y-value (cm)					
		0.5	1.0	1.5	2.0	2.5	3.0
Tetracycline	0.1	+	+	+	+	+	+
	0.10.2						
	0.2	+	+	+	+	+	+
	0.30.2						
	0.3	+	+	+	+	+	+
	0.3						
	0.6	+	+	+	+	−	−
	0.6						
	0.7	+	+	+	−	−	−
	0.7						
Ampicillin	0.1	+	+	+	+	+	−
	0.100						
	0.2	+	+	−	−	−	−
	0.20						
	0.25	+	+	−	−	−	−
	0.25						
Doxycycline	0.3	+	+	+	+	−	−
	0.4	+	+	+	−	−	−
	0.5	+	+	−	−	−	−
	0.6	+	+	−	−	−	−
Amoxicillin	0.2	+	+	+	+	−	−
	0.3	+	+	+	−	−	−
	0.4	+	−	−	−	−	−
	0.5	+	−	−	−	−	−

$n = 3$; +: growth; −: no growth.

Table 2
Results for calculated minimum inhibitory concentrations of antibiotics against *Lactobacillus acidophilus* determined via thermal ink-jet printing and broth microdilution.

Antibiotic	Cartridge Concentration (mg/mL)	Thermal ink-jet printed MIC (μg/mL)	Corrected Thermal ink-jet printed MIC[a] (μg/mL)	Broth microdilution MIC (μg/mL)
Tetracycline	0.60	0.59	1	2
	0.70	0.55	1	2
Ampicillin	0.10	0.12	0.125	0.25
	0.20	0.12	0.125	0.25
	0.25	0.15	0.25	0.25
Doxycycline	0.3	0.29	0.5	1
	0.4	0.31	0.5	1
	0.5	0.29	0.5	1
	0.6	0.35	0.5	1
Amoxicillin	0.2	0.20	0.25	0.5
	0.3	0.23	0.25	0.5
	0.4	0.15	0.25	0.5
	0.5	0.19	0.25	0.5

[a] MICs were rounded off (corrected) to the next incremental doubling dilution for analytical comparisons with broth microdilution.

thermal ink-jet design, bacterial viability was maintained after printing because the temperature rise of the suspension is very small. A simple approach for determination of MICs using ink-jet printing was designed and used to determine the MIC of ampicillin, tetracycline, amoxicillin and doxycycline against *Lactobacillus acidophilus*. The MIC values obtained for the tested antibiotics were within acceptable range in comparison to broth microdilution technique. As well as being a simple method for MIC determination, the use of ink-jetting to print bacteria offers potential in other areas where having precise control of bacterial concentration and location are important, for instance in the development of standard bacterial colonies for testing antibacterial products.

Table 3
Results highlighting the effect of *Lactobacillus acidophilus* inoculum density on minimum inhibitory concentration values.

Antibiotic	Cartridge concentration (mg/mL)	Inoculum density			
		10^5 Colony Forming Units/mL		10^6 Colony Forming Units/mL	
		MIC Y-Value (cm)	Equivalent concentration (μg/mL)	MIC Y-Value (cm)	Equivalent concentration (μg/mL)
Ampicillin	0.10	3.0	0.12	No MIC	–
	0.20	1.5	0.12	2.0	0.16
	0.25	1.5	0.15	2.0	0.20
Tetracycline	0.60	2.5	0.59	3.0	0.71
	0.70	2.0	0.55	3.0	0.83

Table 4
Results showing the effect of contact time on minimum inhibitory concentration values using tetracycline against *Lactobacillus acidophilus*.

Time	Cartridge concentration (mg/mL)	Y-value (cm)					
		0.5	1.0	1.5	2.0	2.5	3.0
0 min	0.3	+	+	+	+	+	+
	0.3						
0 min	0.7	+	+	+	−	−	−
	0.7						
20 min	0.3	+	+	+	+	+	+
	0.3						
20 min	0.7	+	+	+	−	−	−
	0.7						
48 h	0.3	+	+	+	+	+	+
	0.3						
48 h	0.7	+	+	+	+	+	+
	0.7						

$n = 3$.
+: growth; −: no growth.

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Declaration of Competing Interest

None.

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