Genes & Diseases (2017) 4, 163–169

Available online at www.sciencedirect.com

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journal homepage: http://ees.elsevier.com/gendis/default.asp

FULL LENGTH ARTICLE

# A novel method to detect bacterial resistance to disinfectants

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Received 19 June 2017; accepted 2 July 2017
Available online 8 July 2017

**KEYWORDS**
Clinical technology;
Disinfectants resistance;
Inhibitory efficacy;
Oxford plate method;
*Staphylococcus aureus*

**Abstract** In clinical practice, the important hygienic prevention of bacterial pathogen spread is disinfection of potentially contaminated area. Benzalkonium bromide and chlorhexidine acetate are commonly used disinfectants with a broad spectrum of antimicrobial effect. It is vital to inhibit the spread of pathogen in hospital. However, a large number of pathogens with the decreased antiseptic susceptibility have been isolated from clinical samples which showed an increased minimal inhibitory concentration (MIC) against those antiseptics. These resistant pathogens are the major causes for nosocomial cross-infections in hospital. The present study demonstrated the utility of Oxford plate assay system in determining the potential disinfectant resistance of bacteria. The microbiological assay is based on the inhibitory effect of tested disinfectants upon the strains of *Staphylococcus aureus* and *Escherichia coli*. Statistical analysis of the bioassay results indicated the linear correlation ($r = 0.87–0.99$, $P < 0.01$) between the diameter of growth inhibition zone and the log dosage of the tested disinfectants. Moreover, comparison of inhibitory efficacy of benzalkonium bromide upon 29 *S. aureus* strains isolated from clinical samples by both Oxford plate method and broth dilution method showed that the diameter of growth inhibition zone has significantly negative correlation with the minimal inhibitory concentration (MIC) ($r = -0.574$, $P < 0.001$). These results suggest that the Oxford plate is a simple

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Peer review under responsibility of Chongqing Medical University.
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http://dx.doi.org/10.1016/j.gendis.2017.07.001

and time-saving method in detecting potential clinical disinfectant resistance and its usefulness for routine surveillance of pathogenic resistance to disinfectants warrants further investigation.


## Introduction

In clinical practice, the important hygienic prevention of bacterial pathogen spread is disinfection of potentially contaminated area, such as rooms, utensils and hands. Some antiseptics and disinfectants, sharing common characteristics,[1] such as irritation, contact dermatitis, and urticaria, have to be carefully used at minimal bactericidal concentrations in controlling pathogen contamination. For example, povidone-iodine and chlorhexidine are important antiseptics for decreasing skin contaminations of Gram-positive and Gram-negative bacteria, especially those antibiotic-resistant strains, such as MRSA and CA-MRSA. Aldehydes and quaternary ammonium compounds, such as benzalkonium bromide and benzalkonium chloride, are wildly used as disinfectants for sterilization of non-living objects or surfaces.[2,3] The usage of those chemical antiseptics and disinfectants is the vital way to inhibit the spread of pathogen in hospital. However, a large number of pathogens with the decreased antiseptic susceptibility have been isolated from clinical samples which showed an increased minimal inhibitory concentration (MIC) against those antiseptics.[4–8] These resistant pathogens are the major causes for nosocomial cross-infections in hospital. Sometimes, the infection is fatal. Since 1980s, Pseudomonas and *Pseudomonas cepacia* have been isolated in iodophor and polyvinylpyrrolidone-iodine complex (PVP-I) respectively. And the last one has resulted in pseudobacteremia.[1] Similarly, Gram-positive bacteria resistance has also been reported in the past decades. The activity of three *Enterococcus* spp strains can last for 5 min in 100 ppm available chlorine, while only 2 min for non-resistant *Enterococcus* in 0.5 ppm chlorine.[9] Based on these clinical observations it's important and crucial to monitor pathogen resistance against system antiseptics and disinfectants.

To closely monitor the bacterial adaptation and resistance to antiseptics and disinfectants, some microbiological assays used in evaluation of antibacterial activity of antibiotics, such as agar diffusion assay,[10] cylinder plate assay,[11] could be applied. Although less accurate and less precise than HPLC assays, the microbiological assays are still widely used for clinical practice because of their advantages of short turn-around time, simplicity, and low cost. One of the agar diffusion bioassay, Oxford plate assay, is commonly used for the measurements in antibiotics for antibacterial activities and cytotoxicity based on the correlation of the size of bacteriostatic ring and the dosage of antibiotics.[12–16] However, the usefulness of the Oxford plate method in detecting the resistance of pathogen against chemical disinfectant is not well studied. To explore such potential Oxford plate method was used in the present study to determine the potential disinfectant resistance of *Staphylococcus aureus* and *Escherichia coli* against benzalkonium bromide and chlorhexidine acetate in dosage forms of MIC.

## Materials and methods

### Bacteria strains

The *S. aureus* (ATCC6538) and *E. coil* (8099) were routine strains in chemical disinfectant and antibiotic-resistance detection. They were provided by Chinese PLA Center for Disease Control & Prevention, Academy of Military Medical Sciences. The 29 *S. aureus* clinical strains were automatically separated by VITEK 2 Compact (Biomérieux, France) from the Department of Infection Control, Air Force General Hospital.

### Chemicals

The stainless steel Oxford plates with inner diameter (6.0 ± 0.1) mm, outer diameter (7.8 ± 0.1) mm, height (10.0 ± 0.1) mm were purchased from Shanghai Huake Labware Co., LTD. The gradient dilutions of benzalkonium bromide from 50 mg/mL stock solution (Nanchang Baiyun Pharmaceutical) were 32 000, 16 000, 8 000, 4 000, 2 000, 1 000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812 5 μg/mL with $ddH_2O$. And chlorhexidine acetate (Jiutai Pharmaceutical) was dissolved with $ddH_2O$ to a final concentration of 2 000, 1 000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812 5, 3.906 μg/mL. The MUELLER-HINTON (MH) agar plates (90 mm) and MH broth powder were purchased from BIOMERIEUX and OXOID.

### Microbiological assay

#### Oxford plate assay

3–4 bacteria colonies were picked up and resuspended in sterile PBS. The suspension was adjusted to 0.5 McF (equal to $1.5 \times 10^8$ cfu/mL) by turbidimeter and then diluted by 10 folds in which the concentration was around $1.5 \times 10^7$ cfu/mL. The suspension was homogeneously smeared on the MH plate using sterile cotton swab. 2 cylinders were placed on the surface of inoculated medium. The cylinders were filled with 250 μL containing the titrations of benzalkonium bromide or chlorhexidine acetate. After incubation for 24 h at 37 °C, the zone diameters of the growth inhibition were measured and compared. Same experiments were performed with the 29 clinically isolated *S. aureus* with 60 μg/mL benzalkonium bromide. Each concentration listed in 2.2 chemicals was tested for quadruplicates.