

**REPORT ON ANTIBACTERIAL SUSCEPTIBILITY
(DISC DIFFUSION TEST)**

Prepared for

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By

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Antibacterial Susceptibility (Disc Diffusion Test) [(Bauer et al., 1966; Matushchek et al., 2014)]

Methodology:

1. Discs containing antibacterial agent polyhexamethylene cation (PHMB) [test material] were prepared by soaking sterile paper discs in PHMB solution (1000 pm) for 1 h and air-dried for 24 h in a Biological Safety Cabinet at room temperature.
2. Gram positive bacterium (*Streptococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa* & *Escherichia coli*) were cultured using Mueller-Hinton (MH) broth for 24 hours at 30°C/37 °C.
3. The cultured bacteria were streaked on MH agar using sterile cotton swab.
4. Penicillin (10 mg/mL), gentamicin (10 mg/mL), kanamycin (30 mg/mL) and streptomycin (10 mg/mL) antibiotic discs were used as positive control & disc without any agent was used as negative control.
5. Antimicrobial discs were placed onto the streaked agar and incubated for 24 hours at 30°C (*P. aeruginosa* & *S. aureus*) and 37 °C (*E. coli*).
6. After 24 hours of incubation, inhibition zones were measured and recorded. All test were carried out aseptically.

Results:

The results obtained during the experiment are as follows:

Table 1: Inhibition of selected Gram positive and Gram negative bacteria by antibacterial agents.

Agent/ Bacteria	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
	Inhibition zone (mm)		
PHMB	7	8	10
Penicillin	0	0	13
Gentamicin	16	13	17
Kanamycin	0	13	16
Streptomycin	10	10	10
Negative control	0	0	0

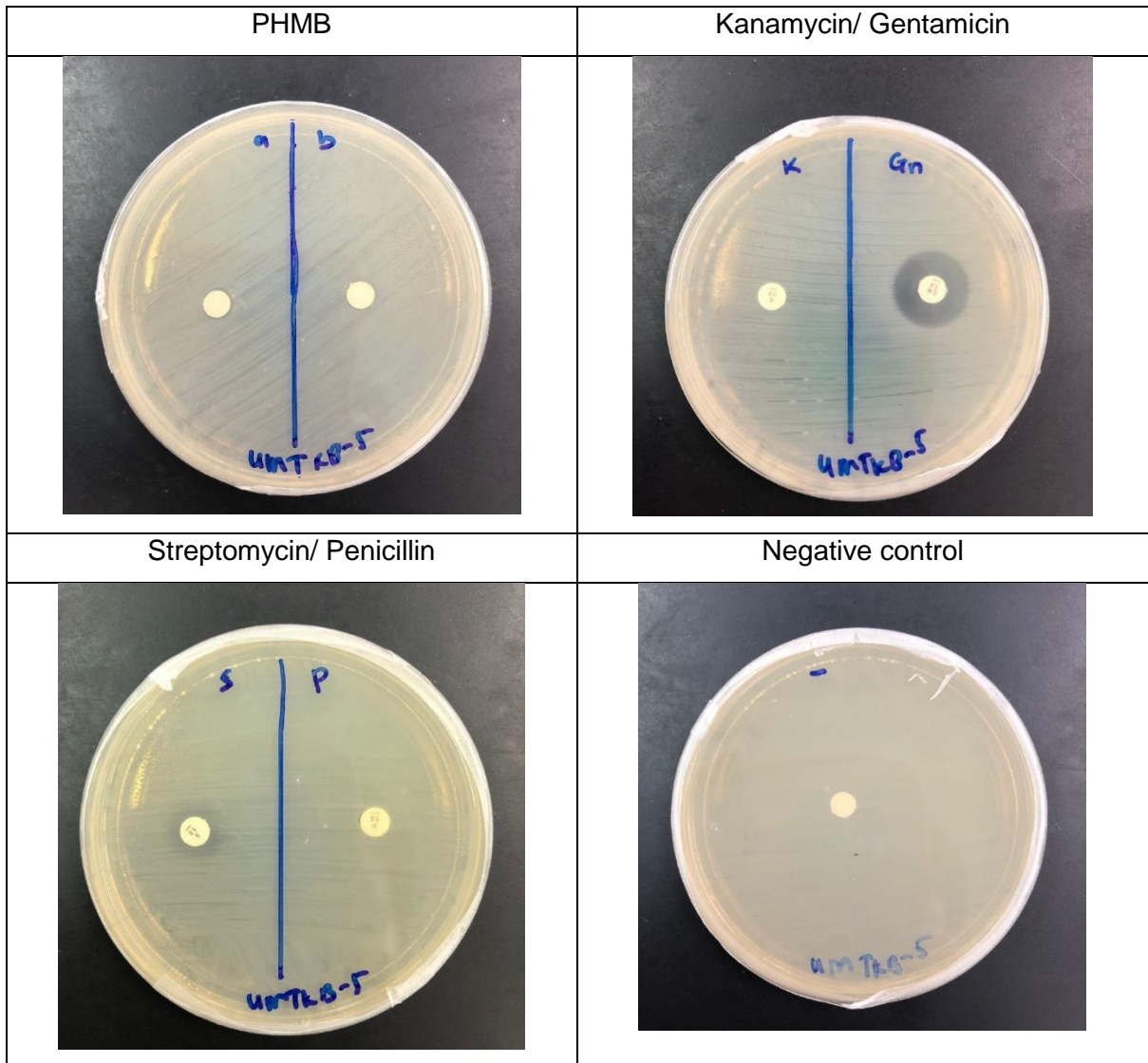


Figure 1: Formation of clear zones which indicates inhibition of *P. aeruginosa* by antibacterial agents. PHMB (a & b), Kanamycin (K), Gentamicin (Gn), Streptomycin (S), Penicillin (P), Control (-).

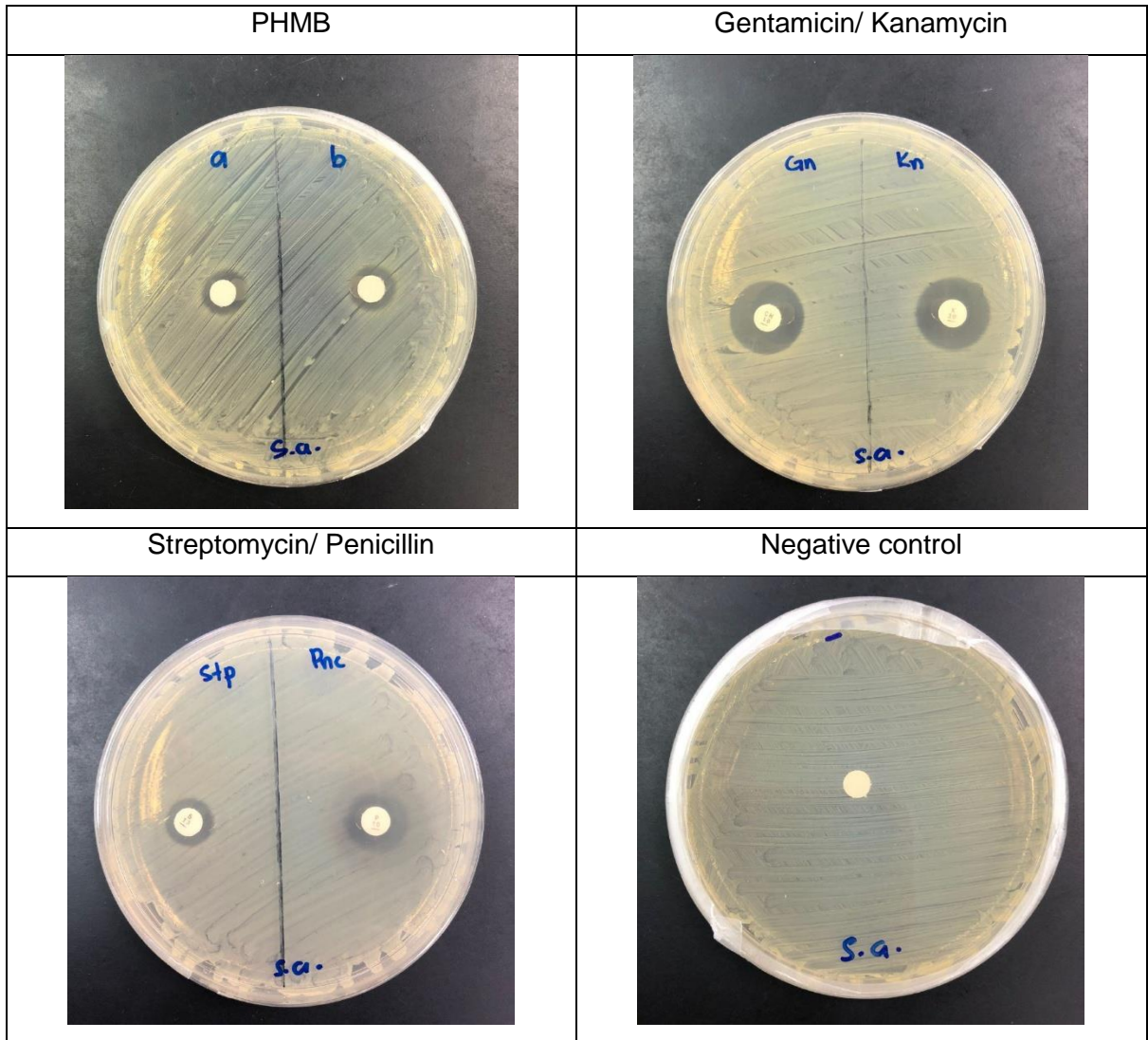


Figure 2: Formation of clear zones which indicates inhibition of *S. aureus* by antibacterial agents. PHMB (a & b), Kanamycin (Kn), Gentamicin (Gn), Streptomycin (Stp), Penicillin (Pnc), Control (-).

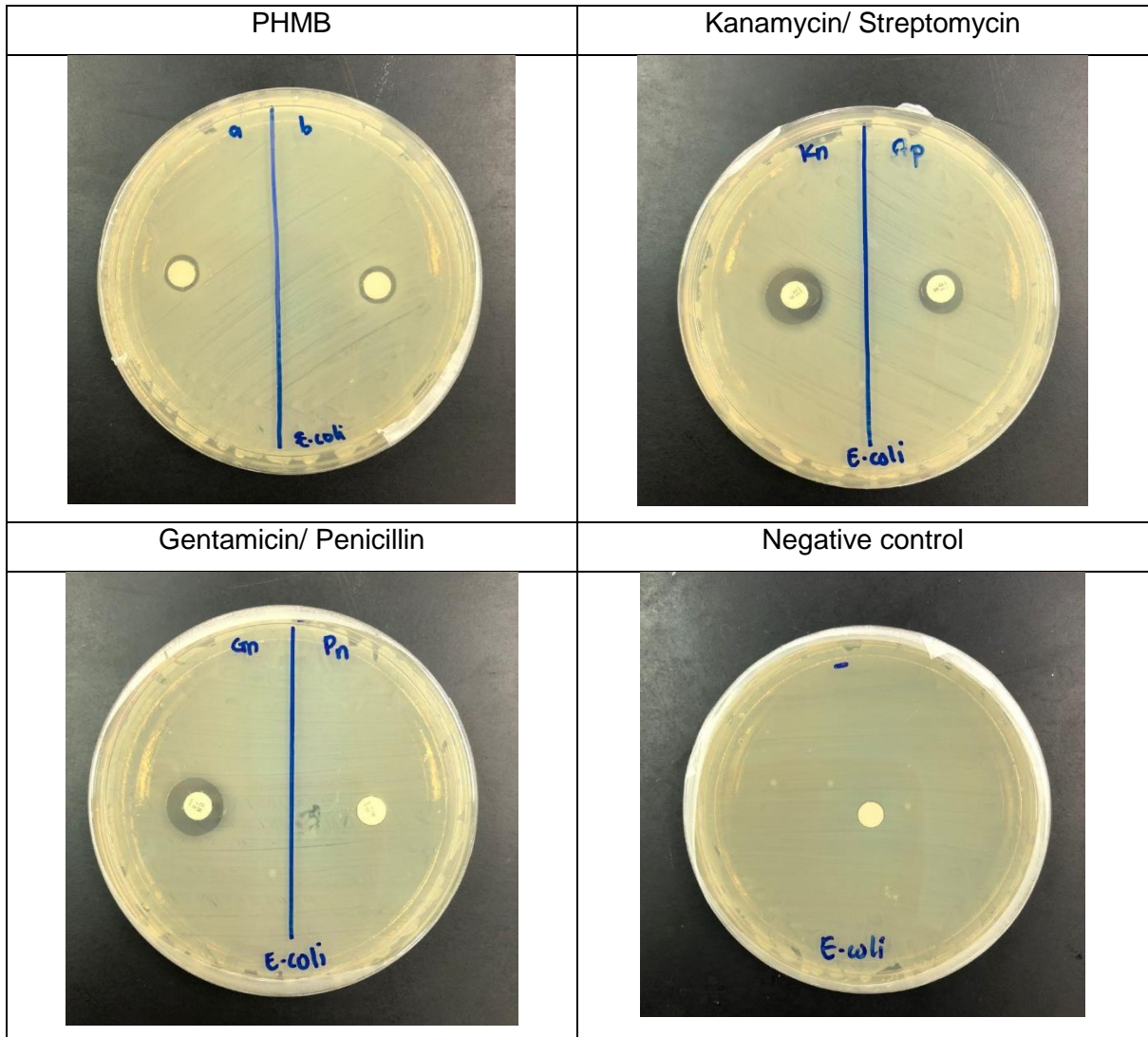


Figure 3: Formation of clear zones which indicates inhibition of *E. coli* by antibacterial agents. PHMB (a & b), Kanamycin (Kn), Gentamicin (Gn), Streptomycin (Stp), Penincilin (Pn), Control (-).

Conclusion:

Perliminary test conducted using disc diffusion method showed that the test material PHMB at a concentration of 1000 ppm exhibited inhibition against the tested bacterial strains, which can be found on human skin. However, the inhibition appears to be lower than the positive control materials which are antibiotic discs. This observation could be due to the presence of higher concentration of antibiotics in the antibiotic discs. The antibiotic discs were used to exhibit positive inhibition effect rather than to measure the effectiveness against PHMB. Nevertheless, it is recommended to further test the effectiveness of PHMB using minimum inhibitory concentration (MIC) assay and against other commonly used similar active ingredients in hand sanitizing solutions currently available in the market.

References:

- Bauer, A. W. , Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45 (4_ts), 493-496.
- Matuschek, E., Brown, D. F., & Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection*, 20(4), 255-266.

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